SYSTEMATIC REVIEW OF POSTMORTEM EXAMINATION IN TOXICOLOGICAL FATALITIES

BY

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ABSTRACT

Postmortem examination in intoxicated deaths is required to establish the cause and manner of death. It relies mainly on the identification of drugs or their known metabolites. This review provides a comprehensive account of toxicological fatalities involving investigative data, autopsy findings, analytical methods, and toxicological results. Postmortem diagnosis of fatal intoxications is complicated. Autopsy findings indicative of an intoxication-related death are scarce and mostly unspecific. Instead, the diagnosis is more dependent on the circumstances surrounding death, and on the toxicological results. The collection of specimens is the first and most important step in the toxicological examination as the proper biological specimens can maximize the chance of obtaining meaningful analytical findings. Since deterioration of specimens increases with postmortem time interval, biological specimens should be collected as soon as possible after death. Additionally, the collection of specimens after the autopsy is rarely possible. To correctly evaluate the toxicological results, the forensic investigator needs to separate between the lethal and non-lethal concentrations. While a wealth of scientific data exists concerning concentrations and effects in experimental animals and in living human subjects, these data cannot simply be translated into postmortem concentrations. Due to various changes occurring in the early phase after death, the postmortem concentration of a substance often does not mirror the concentration antemortem.

In conclusion, it is important to correlate the clinical and autopsy findings with the toxicology results to appropriately evaluate toxicological deaths. Postmortem reference values are needed, and more studies should be hired for the establishment of postmortem reference concentrations.

Keywords: *Autopsy, Intoxication deaths, Sampling, Reference values.*

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1. Introduction

The legal basis for a medicolegal cause of death investigation differs between countries. The investigation is usually started by the police, who seek the expertise of the authority in charge of forensic medicine, often resulting in an autopsy performed by a forensic pathologist or a medical examiner ***(Hanzlick, 2007)***. The medicolegal autopsy rate varies greatly between countries, from a few percent to over 20% of all deaths. Likewise, the rate of postmortem toxicological investigations depends on a multitude of factors. These include differences between types of postmortem cases and between requests from individual forensic pathologists and medical examiners ***(Ojanperä and Vuori, 2014)***.

The main indications for postmortem toxicological investigation are suspicions, circumstances, or autopsy findings that suggest poisoning. A toxicological investigation should also be performed in cases of homicides, traffic accidents, occupational accidents, suspicions of malpractice, and where the immediate cause of death is unknown following an autopsy. The deaths of known drug and alcohol abusers are often associated with accidental or suicidal overdoses. A toxicological investigation is desirable in all cases involving children and young adults, regardless of their apparent cause of death **(Di Candia *et al.*, 2022)**.

Postmortem toxicological investigations have traditionally been used to resolve individual cases. However, postmortem toxicology can serve society in a broader sense by contributing to death statistics and scientific studies, as in studies on drugs related to motor vehicle crashes **(Mørland *et al.*, 2011)**.

A precise post-mortem examination is a fundamental part of the evaluation of toxicological death cases since a comprehensive evaluation of clinical, circumstantial, toxicological, and autoptic data is the only possibility to assess the toxicological significance of a substance and to identify a probable mechanism of death, which could remain unclear despite an exhaustive analysis of all data existing ***(Giorgetti et al., 2020).***

2. Historical background

According to the history of poisons written in an Egyptian manuscript, Ebers Papyrus, approximately 1500 BC, mandrake, hemlock, opium, aconites, and certain metals from natural sources were known for their poisonous properties and they had been used as weapons or in torture. The Greeks used hemlock as a means of state-sponsored execution, Socrates being the most famous case ***(Levine, 2020)***.

A poison can be defined as any substance that when taken in a sufficient quantity will cause intoxication or even death. This dose-dependent relationship was first explained by Paracelsus (1493–1541) with the statement "Sola dosisfacitvenenum" (only dose determines the poison). For instance, even the well-known deadly poison such as cyanide (CN), arsenic, or carbon monoxide may not be harmful if it is inhaled or ingested in a minute quantity. On the other hand, substances as harmless as drinking water or minerals such as potassium or sodium, if taken in excessive quantity, could induce death ***(Cheng, 2009)***.

In 1814, Dr. MattheiuOrfila, a Spanish-born French physician, and the chairman of the Legal Medicine Department at the Sorbonne made the first attempt to systematically study and categorize poisons. In his book "Traité des Poisons ouToxicologieGenerale", he established six classes of poisons, basing the six classes mainly on their toxic effects. The use of postmortem analysis as crucial evidence in court can be dated back to 1840 when Dr. Orfila, was invited by the court to investigate the case of Marie LaFarge for the murder of her husband using arsenic, a common poison used in those days.

Before Orfila's investigation, toxicological analysis of arsenic was found positive in the food but was not detected in the stomach content of the victim using the Marsh test. However, Orfila discovered that the test had been inappropriately performed, and arsenic was later detected in the victim's body. Consequently, Marie was found guilty of murder ***(Bertomeu-S´anchez and Nieto-Galan, 2006).***

In 1851, Jean Servais Stas developed the first effective method for extracting alkaloids from biological specimens. Specifically, his method detected nicotine in postmortem specimens obtained from Gustave Fougnies, who was allegedly poisoned by his brother-in-law. The extraction procedure used by Stas was modified several years later by F.J. Otto. This method, which enabled the isolation of purer alkaloid substances, became known as the Stas–Otto method and remains the basis for drug extraction to this day ***(Levine, 2020)***.

To analyze postmortem samples for a wide variety of potential intoxicants, which are unknown to the toxicologist in most cases, is a challenging task.

3. Postmortem toxicology challenges

To analyze postmortem samples for a wide variety of potential intoxicants, which are unknown to the toxicologist in most cases, is a challenging task. Moreover, there are several other factors ***(Söderberg, 2019):***

- Since postmortem toxicology deals with deceased subjects there is often a lack of information; the initial suspicion for a drug in the first place is largely dependent on the **circumstances** provided to the pathologist it might not be known how much of a given drug was ingested (and not even which drugs are suspected). Hospital records might be lacking or unavailable. Circumstances surrounding the death might be unknown or unclear.
- The focus of the autopsy is both to exclude a morphological cause of death and investigate pathological consequences of detected drugs and thus to provide guidance in an investigation
- One of the most important purposes of the autopsy is to **obtain samples** for further investigation
- The toxicology findings may not be reported for **several weeks** after the body itself has been interred or cremated and the laboratory results may be **non-contributory**.

4. Medicolegal applications of postmortem toxicological analysis

According to **Levine (2020),** apostmortem toxicological analysis is needed in death cases where:

a. The direct cause of death is suspected to be drug or poison-related e.g., intravenous drug deaths with observable recent injectanalysesion sites and oral intoxications indicated from a large number of unabsorbed tablet fragments in the stomach

b. The cause of death is suspicious or unknown, a toxicology laboratory analysis is needed to identify and quantify the substances present in the biological specimens in order to establish a cause and/or manner of death (including drug or poison-related).

c. Deaths other than drug intoxication, such as homicides and accidental deaths. A toxicological analysis is needed to clarify the manner of death, for the following reasons:

- Many homicides are drug-related and the abuse of drugs may provide a motive for the homicide.
- In fatal traffic accidents, ascertain any influence of alcohol and/or drug(s) on the driver.
- In arson deaths postmortem carbon monoxide analysis can also be relevant.

d. Involvement of drugs or poisons needs to be ruled out to support negative pathological findings during autopsies;

e. Toxicological analyses may even be important in deaths due to natural causes, as detected drugs may give clues as to any underlying disease process, i.e., anticonvulsant drugs, antidiabetic drugs, etc.;

f. When there is a statutory requirement for autopsies and subsequently toxicological examinations are deemed necessary by an authority.

5. Role of the autopsy in toxicity-related deaths

According to **Ojanperä and Vuori (2014),** postmortem examination is mandatory in drug or poisoning deaths to establish:

- Whether death is related to a drug or toxin or other cause (e.g. positional asphyxia/pneumonia, or a combination of both).
- The pathological effects of drug or toxin use or misuse.
- If any traumatic injuries were a consequence of previous drug use.
- If there was any natural disease that might have increased susceptibility to the effects of a drug or toxin.
- If the toxicity could have been treated such as to prevent death.
- And to obtain suitable samples for toxicological analysis.

6. Information required prior to autopsy

According to guidelines of **The Royal College of Pathologists (2018),** before undertaking a post-mortem examination, the pathologist should be briefed on any available information. As the initial approach to the postmortem examination will depend on any information provided and it is vital that the final report contains relevant details of the history of the case and the source of the information.

6.1 Scene of death

This should include:

- full details of the scene of death (indoors/outdoors, temperature, exposure).
- how the body was found
- security of the scene
- place, posture, and clothing of the body
- presence/absence of needles, syringes, medicine containers, and pills
- provisional description of the body, including injuries (if any)
- identity of a person discovering the dead person.

6.2 Circumstances of death

This should include:

- witness statements.
- previous medical history.
- medical therapy regimen – current and prior.
- previous surgical operations and other interventions.
- alcohol usage ± illicit drug use.
- previous detention and date of release from prison.
- if there are multiple deaths, the circumstances found at the scene should direct the pathologist on which examinations are appropriate, which may differ between the bodies, e.g. one death may be drug-related, the other traumatic
- known or suspected blood-borne virus status, e.g. HIV, HBV, HCV
- family history.

• electrocardiogram (ECG), enzyme results and other pathological data, serum lipid profiles, and other biochemical tests.

6.3 Possible sources of this information

Important information (and samples for further analysis) may be available from a variety of sources. However, common sources of this material are police, coroner's officers, ambulance notes, general practitioners, hospital clinical notes, laboratory results, family members, and friends.

7. Health and safety precautions

As stated by ***Saukko and Knight (2015a)***, mortuaries have their own local guidelines for dealing with potentially hazardous or infectious cases and the approach taken in suspected drug deaths will vary in line with these. In all cases, the pathologist conducting the post-mortem should assess the risks posed by the case and ensure the post-mortem is conducted in such a way as to minimize any risk to the pathologist themselves and to all other parties involved. Risk assessment is crucial, and the use of personal protective equipment is mandatory. Adequate mortuary ventilation is also required and the use of downflow mortuary tables is recommended for high-risk cases.

7.1 Infections

It should be remembered that intravenous drug users (IVDUs) are at an increased risk of hepatitis, HIV, and tuberculosis, as well as opportunistic infections if their immune system is compromised.

7.2 Chemicals

According to the guidelines of ***The Royal College of Pathologists (2018)***, many industrial activities involve the use of toxic chemicals. In addition, a variety of chemicals can be purchased and used for various purposes including suicide. These agents may be colorless and odorless. A high level of suspicion is needed to detect them before mortuary staff or others are exposed to lethal levels.

In particular, if there is a history of cyanide ingestion or exposure to hydrogen sulfide, extreme caution is required as the cyanide is converted to the poisonous gas hydrogen cyanide in the stomach, which may be fatal if inhaled. It is worth noting that not everyone can smell cyanide.

In cases involving toxic chemicals, the possibility of environmental contamination should also be considered.

However, if there is any doubt that the post-mortem can be conducted and the body disposed of in a suitable way, the post-mortem should not be conducted, and the case should be referred to an appropriately equipped mortuary with the correct expertise to deal with such a case.

8. Imaging

8.1 Post-mortem imaging

In postmortem toxicology deaths imaging help to determine the possibility of body packing, bone dense metaphyseal bands of lead lines, for the documentation of trauma, or for other reasons peculiar to any particular case may be indicated in suspected drug-related deaths ***(Usui et al., 2017)***.

In addition, the role of post-mortem cross-sectional imaging (PMCSI) is expanding as experience and expertise in this field develop. There is clear evidence to support the use of PMCSI in suspected drug-related deaths. If the history, scene examination, external examination, and laboratory results as well as the PMCSI images together support a diagnosis of drug-related death, then such a cause of death may be provided without the need for an invasive post-mortem ***(Burke et al., 2012*** *and* ***Winklhoferet al., 2014).***

8.2 Photography

It is highly desirable to have facilities available to photograph any findings of particular interest ***(Saukko and Knight, 2015a)***.

9. Postmortem examination

Fatal intoxications are often difficult to diagnose based on autopsy findings alone. Known clinical symptoms might provide useful information, but they can be unspecific or masked by disease. The vast majority of poisonings with pharmaceutical drugs will leave no characteristic findings at autopsy. Indeed, the most common findings are organ congestion and pulmonary edema, which are quite common in all kinds of deaths ***(Söderberg, 2019).***

There are exceptions though. Residues of powder or colored material might indicate

tablet or capsule remains. Cyanide has a smell of bitter almond. Carbon monoxide poisoning causes a cherry red to light red color of the blood and hence also the livores ***(Skopp, 2010)***. Noncardiogenic pulmonary edema resulting in heavy, enlarged lungs stiff with edema and congestion along with froth in the airways is typically found in opioid toxicity deaths. Liver necrosis can be the result of paracetamol (acetaminophen) intoxication ***(Bessems and Vermeulen 2001)***. The unexpected finding of pulmonary embolism in a young subject may raise suspicion of an effect of antipsychotics, and in the past combined oral contraceptives, both known to increase the risk of thromboembolism ***(Hedenmalmet al., 2004*** *and* ***Jonsson et al., 2008)***, and a massive spontaneous brain hemorrhage, or gastrointestinal bleeding may indicate intake of anticoagulants. These autopsy findings might seem specific, but they can rarely be used in isolation to make the correct diagnosis in the vast majority of intoxication cases **(Table 1)** ***(Crowther and Warkentin, 2008).***

The Royal College of Pathologists (2018) established guidelines for the autopsy when drugs or poisoning may be involved as follows:

9.1 External:

9.1.1 Clothing

Ideally, clothing should be left in situ. However, this is often not the case in practice, particularly if the deceased has been admitted to the hospital prior to death. Any clothing should be documented, and a note made of any drug stuff in pockets or on the person. Be careful when checking the pockets as needles may be present.

9.1.2 External examination

Once items of clothing are removed, a thorough external examination is required to look for signs of intoxication, as well signs of recent and chronic misuse of drugs **(Table 2**).

9.2 Internal

Complete evisceration and examination of the organ systems should be conducted as standard.

9.2.1 Cardiovascular system

- Dilated cardiomyopathy (ethanol).
- Infective endocarditis (right-sided infective endocarditis in intravenous drug users).

9.2.2 Gastrointestinal system

- Invert the esophagus to look for pills or signs of lacerations from violent retching (Mallory-Weiss tears). Varices are difficult to demonstrate post-mortem owing to the collapse of venous circulation.
- Chronic hemorrhagic gastritis is a well-known consequence of ethanol abuse. It is unlikely that the ingestion of medications will cause marked gastric changes as these drugs are often designed to minimize such effects. By contrast, ingestion of chemicals such as paraquat will often result in marked necrosis and inflammation of the mucosa.
- The pancreas may show signs of acute hemorrhagic pancreatitis (a potential cause of death) or chronic pancreatitis, often owing to chronic ethanol abuse. In practice this is often difficult to assess owing to hemorrhagic autolytic changes and histology is recommended if there is doubt.
- The liver may be obviously steatotic or cirrhotic in cases of chronic hepatitis or alcoholic liver disease. It is not possible to rule out more uncommon causes of liver disease macroscopically and histology should be taken where possible to rule out more unusual diseases such as hereditary hemochromatosis, particularly in at-risk populations.
- The intestines should be fully opened whenever ‘drug packing’ might reasonably be suspected (custodial deaths, death in a nightclub or recent travel from another country).

  Assess for any evidence of mucosal discoloration.

  When removing the intestines, check for segments of infarcted bowel (due to hypotension or emboli).
- Other potential findings include:

- Consequences of cirrhosis (and subsequent portal hypertension) include:

(Splenomegaly, oesophageal varices, spontaneous bacterial peritonitis)

- Increased cancer risk associated with chronic alcohol abuse (hepatocellular, oesophageal, oral, pharyngeal).

9.2.3 Central nervous system

- The head should be opened and examined in all cases to exclude trauma or occult bleeding and to demonstrate hypoxic change. This

includes the examination of the sinuses and dural stripping.

- The brain may show cerebral edema demonstrated by increased weight, flattening of gyri, and filling of sulci. If edema is extreme, a herniation may occur.
- Other potential findings include:
- abscess, meningitis, mycotic aneurysms, empyema (subdural or epidural)
- cerebellar atrophy
- bilateral symmetric necrosis of the globus pallidus (associated with heroin)
- subdural hemorrhage (trauma)
- subarachnoid hemorrhage (if pre-existing berry aneurysm /weakness)
- Wernicke-Korsakoff syndrome (thiamine deficiency in alcoholics; mammillary body atrophy and hemorrhage).
- central pontine demyelination (associated with rapid rehydration and hyponatremia).

9.2.4 Musculoskeletal system

Intoxicated individuals are more prone to trauma and are at higher risk of assault. Possible findings include:

- fractures
- osteoporosis
- infectious spondylitis and sacroiliitis (IVDU)
- thrombophlebitis (IVDU)
- myositis ossificans in the brachialis muscle (IVDU).

9.2.5 Respiratory system

- Removal of the tongue along with the other neck structures is important; look for signs of tongue biting (seizure activity), airway obstruction, and gross congestion of the pharynx (anaphylaxis).
- The lungs may show massive pulmonary edema, characterized by increased weight (weight pre-dissection). There is an increased tuberculosis risk. Also, pneumonia is associated with chronic alcohol abuse.

9.2.6 Genitourinary system

The bladder should be removed and examined in all cases; urinary retention is associated with psychoactive substances, such as Methylenedioxymethamphetamine (MDMA) or Ecstasy, and incontinence is associated with seizure activity.

Other potential findings include:

- bladder distension (MDMA)
- urinary incontinence (seizure activity)
- urinary retention (anti-cholinergic drugs)
- hemorrhagic cystitis (ketamine).

10. Sampling

10.1 Toxicological sampling

Unlike in clinical toxicology, where serum/plasma and urine are usually available, the choice of specimens in postmortem toxicological investigation can be extensive and variable. The specimens selected for analysis can vary depending on the case and the information provided to the pathologist during the investigation. Additionally, the instrumentation and methodologies available to the laboratory will also determine what a laboratory can do. Importantly, the specimens obtainable will play a major role, since some specimens may not be available such as urine if the bladder is emptied or because of decomposition. Generally, the pathologist has the final say on the specimens collected. However, toxicology samples are best taken before any significant disruption of the body has occurred from the autopsy, even if it is later decided that toxicology testing is not required ***(Skopp, 2004).***

The most common specimens used for general toxicological examinations of drugs and poisons in postmortem cases are blood, urine, and vitreous humor. When these are not available, tissue samples such as liver, brain, lung, muscle, and bones and body fluids such as bile, pleural effusions, and other specimens (e.g., hair and nails) are all useful in postmortem analysis **(Table 3)**. The name of the individual who collected the samples and the sites from which each sample is taken must be recorded. All specimens collected should be stored in tightly sealed containers at low temperatures (usually below 4°C for short-term storage during analysis and −20 °C for longer-term storage) ***(Cheng, 2009).***

10.1.1 Blood

Blood is often the specimen of choice for detecting, quantifying, and interpreting drug concentration in postmortem toxicology as most of the literature data are based on their examination in blood. Drug concentration in blood is useful for establishing any recent ingestion of the drug in question and to determine the effect of the drug on the deceased at the time of death ***(Wineket al., 2001).***

The blood sample is preferentially taken from peripheral sites such as the femoral (upper leg) or subclavian region rather than from the cardiac region in order to avoid contamination from abdominal fluids and contents and to reduce artifactual rises in blood concentration due to postmortem redistribution. However, diffusion of drugs from the urinary bladder to femoral blood can take place if a large amount of urine contains a high concentration of the drug **(*Moriya and Hashimoto, 2001)***. At least 10 ml peripheral blood (if not be available, as much as practical has to be accepted). Sodium fluoride/potassium oxalate (preferably 2% w/v) should be used as a preservative to suppress the postmortem production of alcohol, γ -hydroxybutyrate, cyanide, and carbon monoxide and reduces hydrolysis of some drugs, such as cocaine to benzoylecgonine ***(Saukko and Knight, 2015b)***.

If vascular blood cannot be obtained, sampling can be made from the thoracic or the abdominal cavity. However, the composition of these samples will be markedly different from the whole blood and should therefore only be used to qualitatively determine the presence of drugs or poisons. In case of advanced putrefaction, the pleural fluid should be collected for screening ***(Skopp, 2004)***.

10.1.2 Urine

Urine is a convenient specimen for toxicological screening. This is because (i) a relatively high concentration of drugs and their metabolites accumulate in urine and (ii) the drug detection time window in urine is usually longer than that in blood, thereby facilitating the detection of any possible exposure to the potential drug(s)/poison(s). Immunoassay can be performed directly on urine specimens for the detection of certain drug classes, especially for those commonly abused drugs. However, there is no correlation between urine drug concentration with pharmacological effects because of the time difference between drugs absorbed into the bloodstream and drugs eliminated into the urine. In addition, in acute drug-related deaths where survival time (probably less than 15min) is short, the drug may not be excreted into the urine. Therefore, when both urine and blood are available, blood cannot be substituted by urine for the screening of drugs and poisons, and if found, the quantitation of drugs or poisons should preferably be performed on blood. Unfortunately, urine cannot be collected in cases due to perimortem voiding or decompositional changes ***(Jones, 2012).***

Urine should be collected by creating an incision in the upper anterior fundus, or by aspiration with a 20 ml needle and syringe. If practical, at least 20 ml (>10 ml) of urine should be collected in post-mortem cases. The use of fluoride as a preservative is encouraged ***(Saukko and Knight, 2015b)***

10.1.3 Vitreous humour

Vitreous humor is the major fluid component of the eye, and is well protected inside the eyeball so that it is less subject to contamination and bacterial action and has little protein content. In conjunction with alcohol levels in the blood and/or urine, quantification of alcohol in vitreous humor is useful to assist in distinguishing between alcohol intake before death and postmortem alcohol formation. In addition, this specimen is particularly useful for determining common antidepressants, digoxin, sodium, chloride, glucose, and metabolites related to renal functions (e.g., urea nitrogen, uric acid, and creatinine). Thus, it should be collected whenever possible ***(Levine, 2020).***

All vitreous humour from both eyes should be collected (~2-5 ml); Following removal, the shape of the eyes can be restored by injecting water ***(Cheng, 2009).***

10.1.4 Liver

The liver is the major organ for detoxification. Many toxic substances are present in the liver in higher concentrations than in the blood. It is easily collected and can be readily homogenized. Consequently, the liver is used to supplement the blood concentration data, or may also be the only specimens available in cases of advanced putrefaction. Usually, the liver from deep within the right lobe (>100 g) is preferred to avoid the possibility of gastric diffusion the rom stomach or from mesenteric circulation ***(Cheng, 2009)***. The liver should be finely diced and homogenized in water or a dilute

buffer with a minimum water/buffer-to-liver ratio of 1: 1. Unfortunately, quantitation is hampered by poor databases of reference values ***(Musshoffet al., 2004).***

10.1.5 Gastric content

As oral ingestion remains a common route of drug administration, gastric contents are important to investigate potential poisoning and in case of overdose or acute poisoning, high concentrations of drugs or poisons will be detected. In many cases of acute poisoning, undissolved capsules or tablets may be discovered through visual inspection of the contents, allowing relatively simple drug or poison identification ***(Jones, 2012)***.

The entire volume of gastric contents should be collected and measured. As the total amount of a drug or poison present in the gastric contents is more important than its concentration. When supported by blood and/or tissue findings, a large quantity of the parent drug in the gastric contents as compared to the prescribed dose would indicate drug overdose. Recent intake of strong alkali or acids prior to death can easily be determined by pH measurement. In addition, an alkaline pH may also be due to the ingestion of cyanide, and screening tests for cyanide, such as the Ferroin test, should be considered. The presence of a characteristic odor in gastric content can be a useful indicator for certain toxic substances **(Table 1)** ***(Cheng, 2009)***.

10.1.6 Bile

In the absence of urine, bile can be useful for screening of drugs and poisons as a number of drugs, such as morphine, benzodiazepines, and their glucuronide metabolites, ketamine, etc., are present in higher concentrations in bile than in blood. All available bile should be collected (at least 5 ml or whole gall bladder collected**)** ***(Levine, 2020).***

10.1.7 Other samples

a- Lung tissue

Is particularly useful in the analysis of volatile substances, such as hydrocarbons and other solvents or gases. Approximately 50 g (2 cm cubed) is collected and sealed in a glass airtight container ***(Jones, 2012)***.

b- Brain

Is useful for the detection of drugs such as antidepressants, narcotics, and halogenated hydrocarbons that act primarily on the central nervous system. In deaths due to chloroform poisoning, a high chloroform concentration can be found in the brain. *Approximately 50 g is collected* ***(Cheng, 2009)***.

c- Kidney

Is useful in the investigation of heavy metal poisoning. The high concentration of metal deposited in the kidney is often associated with structural damage that *may be characterized histologically* ***(Jones, 2012*)**.

d- Hair analysis

As the growth rate of hair is approximately 0.6–1.4 cm (about 1 cm) per month, it provides a longer drug surveillance window, on a scale of weeks to months, than that of urine and blood, thus hair samples may be of limited value in determining whether drugs have been taken in the few days prior to death. Hair is used to evaluate prior exposure to heavy metals, such as arsenic, lead, and mercury, and is now extended to the analysis of a wide range of organic drugs and poisons to provide information on the chronic use or long-term exposure to toxic substances **(Kintz, 2004)**.

More than (50 mg) should be collected before the body is opened to avoid contamination of the hair with body fluids. The sample should be cut from the posterior vertex region of the head, as close as possible to the scalp, since this is the region of least variation in growth rate. Identify the distal and proximal end by tying a piece of cotton or string around the hair at that end, then wrap it in an inert covering such as aluminum foil ***(Cheng, 2009)***.

e- Bone marrow

May be analyzed qualitatively where only skeletonized remains are recovered ***(Jones, 2012).***

f- Muscle

Can be useful for screening but quantitations are hampered by poor databases of reference values. The psoas muscle is normally used ***(Levine, 2020).***

g- Injection site (skin)

Injection site (skin) – may be useful in determining the type of substance that has

been injected, such as insulin or heroin. Always send a control site sample for comparison. To sample the injection site, excise a wide skin ellipse, down to subcutaneous tissue. Place the specimens in clean, labeled containers ***(Jones, 2012).***

10.1.8 Antemortem Specimens

When an individual is treated in the hospital prior to death, the collection of these specimens by the postmortem toxicology laboratory may be vital in the cause of death determination. In drug intoxication cases, metabolism and medical intervention may cause a significant decrease in drug concentration or even the removal of the drug from the blood. Moreover, administration of drugs in the hospital for palliative care, such as morphine, may be detected in the postmortem specimens but may be unrelated to the cause of death. However, there are also limitations in the use of these specimens in death investigation cases. Specimen volume is often limited, preventing the performance of a comprehensive drug screen. For that reason, it is best to only test the antemortem specimens either after a screen on the postmortem specimens or after consultation with the pathologist so as to target the testing that is needed ***(Levine, 2020).***

Table (1): Autopsy findings indicative of intoxication or poisoning *(Skopp, 2010)*

Findings	**Indicative of**
***Odor**	
Bitter almond	Cyanide, hydrogen cyanide, nitrobenzene
Fruity, aromatic	Ethanol, solvents
Like leek or garlic	Organophosphorus compounds, arsenic,phosphorous
Antiseptic	Chloroxylenol
Sweet	Chloroform or other halogenated hydrocarbons
***Orifices of the body, e.g. mouth or gastrointestinal tract**	
Residues of powder or colored material	Tablet or capsule remains (e.g. flunitrazepam - blue-stained gastric contents), herbicides or pesticides, intranasal drug use (e.g. cocaine)
White, corrosive staining	Hydrochloric or acetic acid
Black-brown, corrosive staining	Sulphuric acid
Glass-like, reddish necrosis	Alkaline agents, e.g. sodium hydroxide
***Lividity**	
Cherry red to light red	Carbon monoxide
Bright pink	Cyanide
Greyish to brownish	Nitrate, nitrite, aniline
***Skin**	
Atrophic scarring, abscess and ulceration of the skin, puncture marks	Intravenous drug use, e.g. opiates
*** Nose**	
Perforated nasal septum	Intranasal cocaine misuse

Table (2): External examination findings suggestive of intoxication *(The Royal College of Pathologists, 2018)*

	Possible finding
a- General	The following should be noticed: • Malnourished, frowzy appearance. • Recent injury. • Needle puncture marks. • Chronic injection sinuses. • Evidence of previous/current self-harm. • Examination of mouth, anus, vagina and under foreskin for evidence of body packing. • Signs of resuscitation (cannulas and ECG stickers) – may explain presence of needle puncture marks. • Skin abscess (skin popping). • Track hyperpigmentation (heroin diluents and adulterants -talc, mannitol, dirt, and clay) • Scars/'homemade' tattoos. • Bright red hypostasis – associated with carbon monoxide poisoning or hypothermia – as well as any other tissue/skin discoloration that may suggest evidence of poisoning. • An abnormal pattern of hypostasis (particularly head or torso dependent) as in postural/positional asphyxiation while intoxicated and/or incapacitate. • Always check the back for any of the above.
b-Chest/abdomen	• Spider naevi (superior vena cava distribution). • Gynaecomastia. • Abdominal distension (ascites). • Bruising/caput medusae. • Haemorrhoids. • Testicular atrophy.
c- Face	• Jaundice (sclera and skin). • Nasal septum perforation (cocaine). • Necrosis of nasal tip (endocarditis due to thrombosis of small vessels as Janeway lesions). • Blood-tinged froth around mouth/nose (pulmonary oedema). • Abnormal coating on tongue. • Foreign body in mouth or nose. • Gum (grey black mercurial line/lead blue lines), Phossy jaw (phosphorus). • Unlike in life, pupil size is rarely of value after death owing to rigor mortis of intrinsic eye muscles.
d- Limbs	• Peripheral oedema. • Erythema over joints (hypothermia).
e- Hands	• Clubbing, nicotine staining, splinter haemorrhages (infected endocarditis). • Dupuytren's contracture (cigarette smoking, alcoholism, medicines used to treat seizures) • Palmar erythema. • Mees' Lines in the nails (heavy metal poisoning)

Table (3): Suggested postmortem specimens to be collected *(Cheng, 2009).*

Type of cases	Specimens collected
General cases	Blood Urine Vitreous humor
General cases (blood not available)	Cavity fluid (for screening) Liver Urinary bladder washing (if urine not available) Vitreous humor (if any)
Drug- or poison-related cases (suicide cases)	Plus, gastric contents
Drug- or poison-related cases (suspicious cases)	Plus, gastric content and liver
Gaseous or volatile substances	Plus, lung and brain tissue
Heavy metal poisoning	Plus, liver, kidney, and hair

10.2 Histological Sampling

Histology is of value in confirming and evaluating the course of natural disease processes. It is important that any natural disease in the deceased is well documented so any possible role it played in the cause of death is known. According to ***The Royal College of Pathologists (2018)*** guidelines, examples of histology and possible findings in a drug death:

10.2.1 Lung (at least one piece per lobe)

• Confirmation of pneumonia versus pulmonary edema (macroscopic inspection is unreliable).
• Aspiration pneumonia, inhalation of vomit, presence, and effect of injected material.
• Emphysematous changes (smoking).
• Marked anthracosis (cannabis).
• Septic pulmonary abscesses.
• Tuberculosis (IVDU).
• Perivascular pulmonary talc granulomas (IVDU).
• Foreign body emboli (IVDU).
• Pulmonary necrotizing angiitis (IVDU).
• Atelectasis, fibrosis (smoking, cannabis).

10.2.2 Kidney (one piece per kidney)

• Glomerulosclerosis, amyloid (IVDU).
• Cocaine' nephropathy (cocaine).

10.2.3 Cerebrum and cerebellum (particularly hippocampus, cerebral cortex, and dentate nucleus)

• Evaluation of hypoxic/ischaemic neuronal damage.

10.2.4 Heart

• Evidence of 'cocaine' cardiomyopathy (cocaine).
• Left ventricle fibrosis, contraction bands, ischaemic heart disease (cocaine).
• Right ventricle – hypertrophy secondary to cor pulmonale (IVDU).

10.2.5 Liver (one piece, away from capsule)

• Assessment of fatty liver/cirrhosis and investigation of etiology (especially in those of a younger age).
• Investigation of hepatitis (viral, alcohol, other).
• Talc granulomas (IVDU).

10.2.6 Additional histology samples according to case

• Skin injection sites (IVDU). The specimen is fixed by neutral buffered formalin. Then examined and serially sliced.
• Quadriceps and psoas muscle, if rhabdomyolysis suspected (ecstasy/opiates).

11. Postmortem toxicology analytical techniques

There are a wide variety of analytical techniques available for the analysis of toxic substances in biological specimens. The most common techniques used in forensic toxicology can be grouped into spectrophotometry, immunoassay, chromatography, and mass spectrometry. Yet, in postmortem specimens, the analytes of interest usually require separation from the biological matrix ***(Cheng, 2009)***.

In cases of drug-related death, "routine" or "comprehensive" toxicology screening

investigations are often carried out, frequently including screening for some of the toxic substances. The methods used may or may not be appropriate for detecting other chemical classes. Therefore, we cannot rely on any single test or screen for every death investigation. The analytical methods selected must be on a case-by-case basis, by considering potential exposures, clinical signs and/or postmortem findings, and many other factors ***(Mogollónet al., 2018).***

11.1 Preanalytical steps

Many analytical methods used by forensic toxicologists require that analytes be chemically extracted from blood, urine, vertus hummer, tissues, gastrointestinal contents, food, and a wide variety of environmental samples ***(Romano et al., 2020)***.

The usual extraction techniques involve either liquid-liquid extraction (LLE) or solid-phase extraction (SPE). The traditional LLE technique is still a common extraction method used in postmortem specimens. The method has the advantage of efficient extraction of drugs and poisons present in a wide concentration range and the absence of adsorption loss frequently associated with a solid surface. Compared with LLE, SPE has the advantages of low solvent consumption, provision of cleaner extracts, ease of automation, and high extraction efficiency for certain specific drugs requiring a smaller sample volume. Thus, it provides an excellent alternative to the traditional LLE for the extraction of postmortem samples ***(Cheng, 2009)***.

Some analytes and chemical methods may require additional preparatory steps, such as the addition of matrix modifiers prior to graphite furnace atomic absorption spectroscopy (AAS) or derivatization of an analyte to increase volatility to facilitate gas chromatography (GC) ***(Maurer, 2018)***.

11.2 Identification

11.2.1 Spectrophotometry

The use of spectrophotometry in today's postmortem forensic toxicology laboratory is limited to some simple color tests and the detection system in some commercially available immunoassays. Color tests are easy to use and can be performed directly on the specimen or on a protein-free filtrate of the specimen. Color tests may be used to screen postmortem specimens for salicylate and acetaminophen, Ferroin test for cyanide, and Marsh test for arsenic ***(Levine, 2020)***.

11.2.2 Immunoassays

Immunoassays use antibodies to bind specific analytes within a sample. They are inexpensive, easy to use, and can be very sensitive. Immunoassays are widely used as screening techniques for commonly abused drugs, such as opiates, amphetamines, cocaine, cannabinoids, phencyclidine, and barbiturates. In addition, prescribed drugs, such as propoxyphene and tricyclic antidepressants, can also be screened with the use of specific reagents ***(Melanson, 2012)***.

A number of immunoassays intended for antemortem analysis can also be used for postmortem analysis especially when urine is available. Homogenous immunoassay can distinguish between bound and free labeled drugs in a mixture, e.g., fluorescence polarization immunoassay (FPIA), cloned enzyme donor immunoassay (CEDIA), and enzyme multiplied immunoassay technique (EMIT). Heterogenous immunoassays, such as enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA), often require a washing step to separate the bound labeled complex from free labeled reagent prior to analysis. Thus, in homogenous immunoassays, direct detection can be achieved without sample purification. However, cutoff values, often applied to workplace drug testing, especially for abused drugs should be used cautiously in postmortem cases since the presence of low drug concentrations can be of forensic significance. In an acute drug-related death, for instance, the drug may not have sufficient time to be excreted into urine before death resulting in a low drug concentration in urine ***(Cheng, 2009)***.

False positives may occur, either from structurally related drugs or from metabolites of other drugs that are recognized by the antibodies. For instance, phenethylamine, a common putrefactive product from decomposed bodies, can cause a false-positive response to the amphetamines class test when using immunoassays. Cross-reactivity can also be due to chemicals with similar

structures to the intended analytes. For example, pholcodine gives rise to a positive response to the opiate reagent of FPIA. Thus, for samples that give positive screening results, confirmation tests should be performed, preferably using chromatographic techniques with MS detection ***(Levine, 2020)***.

11.2.3 Chromatography and mass spectrometry

Chromatographic techniques are used to separate chemicals in a mixture, most commonly through either a gas or liquid mobile phase. They can be coupled with various detectors and used as common instrumentations to screen for a wide range of organic toxic substances. Yet, when combined with mass spectrometry it is a definitive technique to establish proof of unknown substances ***(Peters et al., 2018)***.

Gas chromatography (GC) is useful for volatile, thermally stable, relatively nonpolar compounds. Gas chromatographs are often coupled to various detectors from the more universal flame ionization detector (FID) to specific detectors such as electron capture detector (ECD) and nitrogen phosphorus detector (NPD) or mass spectrometers (MS). FID is useful for the detection of alcohol, other volatile organic compounds, and many other organic drugs and poisons. NPD is sensitive to nitrogen- and phosphorus-containing compounds and is useful for the detection of drugs and poisons, such as antidepressants, antipsychotic drugs, benzodiazepines, opiates, cocaine, and its metabolites, organophosphorus insecticides, etc. ECD is particularly sensitive to halogenated compounds (e.g., chlorinated insecticides), nitriles (e.g., CN), or nitrogen-containing compounds (e.g., benzodiazepines, nifedipine, and zopiclone) ***(Filigenzi, 2018)***.

For confirmation, the high separation power of GC coupled with a highly selective MS detector is regarded as the "gold standard" for the structural identification of drugs and poisons. In GC–MS, electron ionization (EI) is a commonly used ion source. EI applies a standardized electrical current to a filament, ionizing and fragmenting compounds. Which produces a characteristic mass spectral "fingerprint" that can be compared to known standards or mass spectral libraries. By using a well-established and standardized current, EI produces a consistent fragmentation pattern for an analyte. GC–MS libraries are thus shareable between instruments ***(Romano et al., 2020)***.

Liquid chromatographic techniques, including high-performance (HPLC) and ultrahigh-performance (UHPLC), are used for thermally labile, higher molecular weight, and more polar compounds. LC can be coupled to an ultraviolet (UV) spectrophotometric or fluorescence detector, or an MS. Various HPLC and UHPLC methods have been developed for carbamate pesticides, some drugs and their metabolites, mycotoxins, anticoagulant rodenticides, microcystins, and many other compounds. Electrospray ionization (ESI) is often used with LC Compounds that are ionized but remain intact, producing charged ions rather than a fragmentation pattern. Standard LC–MS/MS libraries are not possible because fragmentation patterns of an analyte vary between instruments and laboratory conditions ***(Filigenzi, 2018)***.

11.3 Quantification

That a substance is present does not necessarily mean that it was a cause of death. For this determination, the substances in the relevant specimens must be quantified. Quantification of drugs in blood is most commonly associated with toxicity or lethality. In chromatographic methods, the signal generated by the detector will be proportional to the amount of substance present. By preparing calibrators of known concentration, response factors can be calculated to quantify the analyte in the case specimens. In certain circumstances, the quantification of drugs in tissue may have particular utility. When blood specimens are unavailable, the liver is usually used as a substitute specimen ***(Levine, 2020).***

11.4 Interpretation and reference concentrations estimation

It should be noted that while substances found in excretory fluids such as urine or bile are useful qualitatively, quantification of drugs and poisons in these fluids usually has limited interpretative values ***(Cheng, 2009).***

Whereas, in blood due to changes in drug concentrations that can occur early after

death, reference information about drug concentrations in living subjects is not a reliable source for comparison when evaluating postmortem toxicological results. The forensic community has responded to this problem by building reference values from postmortem material. There are multiple methods of producing reference concentrations through different types of scientific studies. There are several studies to provide postmortem reference concentrations of many compounds in therapeutic, toxic, and even fatal levels have been published, providing support to the forensic pathologist in the evaluation of postmortem toxicological results ***(Launiainen and Ojanpera, 2013, Kraai and Seifert, 2015, Skovet al., 2015, Skovet al., 2016***, ***Jones et al., 2016, Concheiroet al., 2018*** *and****Aucklooand Davies, 2019)***.North Carolina Office of the Chief Medical Examiner (N.C. OCME) provided a guide table for postmortem drug concentrations. The data have been compiled from previously published scientific literature and from prior OCME experience ***(N.C. OCME, 2017)***.

11.5 Systematic Toxicological Analysis

A comprehensive and systematic analysis of the presence of chemical substances of toxicological significance is termed systematic toxicological analysis. The usual practice in toxicological examination begins with the preliminary identification of alcohol and screening of a wide spectrum of acidic, neutral, and basic organic drugs or poisons. If a toxic substance(s) is detected, confirmatory and, if necessary, quantitative testing must be performed. In general, positive identification is achieved using at least two independent analyses and preferably based on different analytical principles. Using GC-MS or LC-MS, confirmation and quantification can be simplified into one single analysis ***(Cheng, 2009)***.

12. Clinicopathological correlation

The Royal College of Pathologists (2018) guidelines stated that, in potential overdose situations, it is important to consider whether the reported symptoms prior to the death match the alleged drug. Just because a drug or its metabolites have been identified does not mean the level is necessarily fatal or excludes the effects of another unsuspected drug. Alternatively, in cases of suicide or accident, the cause of death may be obvious (e.g. hanging or drowning). However, intoxication may have played a role prior to death and, in these situations, may be recorded second to the primary cause of death. Furthermore, natural diseases that arise from drug abuse are numerous. The effects may be the result of cumulative injury (alcohol cirrhosis) or a consequence of a transient effect of the drug (aortic dissection due to transient hypertension from cocaine). It may not be possible to prove such findings resulted directly from drug use; however, it is acceptable to use past medical history and information available at the time of autopsy to make an informed interpretation of a particular autopsy finding. In situations where drug use has resulted directly in impaired consciousness with subsequent complications e.g. aspiration pneumonia, the drug may still be considered a direct cause of death.

13. Artefacts in postmortem toxicological examinations

Each death is unique, and the factors that may alter the effects on and the concentration of a drug in the body are not known in detail in most cases. It must be accepted that artifacts as an integral part of postmortem toxicology frequently interfere with a straightforward interpretation of the analytical results. Some compounds are recognized to purely arise or be significantly altered in the post-mortem period ***(Flanagan and Connally 2005).***

13.1 Enzyme-activated degradation and putrefaction

Enzymes naturally present in the body cause autolysis, while putrefaction is due to destruction by microorganisms. The pH value in blood immediately drops to a minimum of 5.5 after death has occurred, and a decrease in water content can often be observed in blood samples. Hemolysis is seen with most blood specimens, which can be fluid, clotted, or partially clotted. Bacterial contamination and propagation can be particularly severe in postmortem specimens ***(Wyman et al., 2011).***

The breakdown of benzodiazepines can be attributed to chemical hydrolysis or bacterial action. On the other hand, the long-

term stability of estazolam and alprazolam can be explained by the triazole rings in their structures which make these compounds more resistant to hydrolysis. Conversion of morphine glucuronides to the parent drug in postmortem blood is obviously due to residual or bacterial glucuronidase activities ***(Skopp, 2014).***

However, postmortem change is not static but develops over time. This complicates the evaluation of postmortem toxicological results, especially when the postmortem interval is unknown **(*Zilg, et al., 2017).***

13.2 Post-mortem Blood Distribution

The acquisition of toxicological samples at autopsy must follow a few simple rules; it must be the right specimen, from the right site, in the right bottle, with the right preservative, with the right label, and the right clinical information so that the right tests may be performed and the right interpretation of the results be given. It has been recognized for many years and confirmed with several multisite sampling experiments that the concentrations of drugs in the blood vary from site to site in the body. This is considered to be due to the diffusion of drugs along concentration gradients in the post-mortem period. Thus, sampling should be done from peripheral sites away from the stomach and liver. Cardiac and cavity blood should be avoided wherever possible. If the stomach or small intestine has ruptured, then a source outside the peritoneal cavity should be sought ***(Drummer, 2008).***

In general, the extent of postmortem redistribution is thought to be dependent on three key factors; proximity to one or more reservoir organs, putrefactive changes, and the pharmacokinetic properties of the drug (ex. volume of distribution) involved. Femoral blood is the sample of choice as it is the least affected by redistribution ***(Sastreet al., 2017).***

13.3 Alcohol

The problem of failure to put the right sample in the right preservative is illustrated by blood alcohol results. A sodium fluoride sample bottle should be used to try and prevent post-mortem production of alcohol by yeast and alcohol-producing bacteria flora which colonizes the body during the post-mortem period **(Anderson *et al.*, 2011)**. Levels of alcohol generated in the post-mortem period either within the body or the blood bottle may reach surprisingly high values ***(Ehrlich et al., 2010)***.

13. 4 Carbon monoxide

Carbon monoxide testing is an example of ensuring that the right site is sampled. Carbon monoxide is formed during decomposition from hemoglobin and myoglobin by the action of bacteria. Therefore, if body cavity fluid is sampled, levels of greater than 10 percent carboxyhemoglobin may be observed, purely due to post-mortem formation, especially in case of drowning. As smoking may be a common source of carbon monoxide hemoglobin, a cut-off value of 10% has been suggested ***(Sato, 2005)***.

13.5 Cyanide

As with carbon monoxide, cyanide has also been shown to be produced in the post-mortem period and high cyanide levels may occur in unpreserved blood samples ***(Skopp, 2014).***

13.6 Circumstances of the deceased, the cause of death, and resuscitation

Sastre*et al.* (2017) stated thathospital care, resuscitation, and the cause of death can impact the interpretation ofpostmortem drug concentrations. As,treatment with large amounts of intravenous fluids can dilute, or render tissues devoid of, detectable drug concentrations. The pharmacokinetics of critically ill patients with impaired cardiac output, blood pressure, and ventilation in addition to possible acidosis and the effects of the disease, are probably different from healthy populations. The renal disease might affect the excretion of a drug and liver disease might affect its metabolism. Resuscitation attempts can also impact postmortem redistribution moving central blood more peripherally, and therefore influence the measured concentration of a drug in a peripheral blood sample**.** Traumatic deaths with extended blood loss might affect the concentration of drugs, as the body tries to adapt to exsanguination, such as the transfer of extravascular fluid into circulation**.**

Tolerance, implying various adaptations in the response to a drug upon repeated exposures, represents a well-recognized problem in the clinical setting, particularly in the treatment of chronic pain. Development (or loss) of tolerance is also a factor that must be considered in the interpretation of postmortem blood concentrations. One example are opioids, which are known to induce substantial tolerance when used continuously. It has been shown that blood concentrations alone, are not reliable when evaluating possible opioid intoxications. In these cases, recent previous exposure must be confirmed or excluded to evaluate the impact of an opioid substance detected ***(Drummer, 2005)***.

13.7 Drugs in embalmed tissues and exhumation

In postmortem forensic toxicology, there are instances when the only specimens available for testing are embalmed tissues. Analyte concentration in embalmed specimens, formalin-fixed tissues, and formalin solutions generally cannot be considered representative of concentrations present at death. In addition to factors typically affecting postmortem concentrations, formaldehyde concentration, pH, time, and a dilution effect (in embalmed specimens) may all affect drug identification and concentration. Although some drugs appear stable, most experience a decrease in concentration, particularly at higher pH and formalin concentration. The lack of identification of an analyte does not ensure that the drug was not present at death. For some compounds, particularly those with primary or secondary amines, identification of the potential Eschweiler-Clark reaction products may increase the chances of identification of drug use. It is recommended that multiple specimens should be analyzed if available as drug affinity between tissues may vary ***(Spargo, 2020).***

In several instances, exhumations occur in order to collect tissue to be analyzed for possible metal poisoning. In these cases, analysis of the surrounding soil for the metal or metals of interest should be performed to exclude external contamination of the specimens. Furthermore, contamination during the analytical procedure must also be considered (specimen containers, glassware, etc.). Acid-washing containers in 10% nitric acid remove the metal contamination ***(Saady, 2020)***.

14. Negative autopsy

As believed by ***Saukko and Knight (2015a)*** "The absence of injuries, evidence of poisoning, lethal infection or well-recognized natural disease is in itself significant negative evidence."

Many deaths caused by suspected illicit drugs do not show significant pathology at the time of autopsy. Death may be due to apnea, central nervous system depression, biochemical imbalances, or alcoholic cardiomyopathy. These are difficult to demonstrate on histology and if toxicology is negative or not available, the pathologist may be left with a mystery. Negative autopsies are not a sign of failure on the part of the pathologist, just exclusion of any reasonable natural death and documentation of the absence of significant other findings is considered an important negative finding. Of all deaths, 5% are unascertained.

CONCLUSIONS

From the mentioned data, postmortem toxicology often requires the analysis of a wide variety of tissues, fluids, and specimens for various potential toxicants. Interpretation of analytical results is not always straightforward. Analytical results must be interpreted in the context of the entire case, including clinical and laboratory findings.

Therefore,the conclusion that death was caused by intoxication should be based on three pillars: the history and the circumstances surrounding death must be consistent with poisoning, exclusion of diseases or injuries that are inconsistent with life at the postmortem examination, and a concentration which is typically encountered in such fatalities.

The best way to diagnose intoxications is by correct sampling, toxicological analysis, and a reasonable evaluation of the results. However, in order to evaluate the concentration of a substance in a postmortem cause, the investigator needs to know if the concentration is high or low, normal or toxic.

To this end, postmortem reference concentration data are critical.

Even after a comprehensive analysis of clinical, circumstantial, toxicological, and autoptic data, the cause and manner of death remain unclear in some cases.

RECOMMENDATIONS

Additional studies are needed to establish postmortem reference values for as many as possible drugs and toxic substances via investigating a proper number of cases needed to produce stable and reliable reference values.

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