Post-mortem toxicological analysis of hair in violent fatalities: An investigation into long-term drug exposure

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Abstract

Violence-related injuries are a major cause of mortality in the Western Cape (South Africa). Previous research has demonstrated an association between violent mortalities and drug use. Furthermore, long-term drug use has been shown to alter behaviour that may lead to violence. Hence, this study aimed to investigate the effect of long-term drug use on violence-related mortalities. Due to the drug retention properties of hair, it is the gold standard for demonstrating the historical pattern of drug use. Hair samples were collected from 92 violent death cases admitted to Salt River Mortuary (South Africa). A qualitative toxicological analysis was performed in 90 hair samples using a SCIEX X500R QTOF. Variables pertaining to the colour and length of the hairs were recorded. The majority of the hair samples were black (n=79), while others were black and white (n=5), greyish (n=3), light grey and reddish brown (n=1) and strong brown hair (n=1). Various toxicologically relevant substances were detected in 74 cases (82.2 %) in which a total of 54 different substances were detected in hair samples. Acetaminophen was the most prominent licit substance (47%) detected, followed by caffeine (18.9%) and diphenhydramine (12%). Methamphetamine was the most common illicit detected substance (54%), followed by methaqualone (43%). Segmented hair samples showed historical use in 81.2 % of cases. These results show that hair can be used as a supplementary sample during toxicological investigation in violent fatalities in the local context.

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%	percentage
°C	degrees Celsius
cm	centimetre
CNS	central nervous system
FPS	Forensic Pathology Service
g	gram
HCl	hydrochloric acid
HREC	Human Research Ethics Committee
ISTD	internal standard
LC	liquid chromatography
$m.s^{-1}$	meters per second
MDMA	3,4-methylenedioxymethamphetamine
mg	milligram
min	minute
ml	millilitre
mm	millimetre
MRM	multiple reaction monitoring
ng	nanogram
OTC/PRE	over-the-counter and prescription drugs
QTOF	quadrupole time-of-flight
s	second
SA	South Africa

SACENDU	South African Community Epidemiology Network on Drug Use
SoHT	Society of Hair Testing
SRM	Salt River Mortuary
THC	tetrahydrocannabinol
THC-COOH	11-nor-9-carboxy-Δ9-tetrahydrocannabinol
UCT	University of Cape Town
UHPLC-MS/MS	ultra-high-performance liquid chromatography tandem mass spectrometry
WHO	World Health Organization
µl	microlitre

CHAPTER 1: LITERATURE REVIEW

1.1. INTRODUCTION

In South Africa (SA), deaths due to physical or chemical influences, sudden or unexpected deaths, procedure-related deaths and deaths related to an activity of omission or commission are admitted to forensic mortuaries for medico-legal investigation to determine the cause of death (Inquests Act, 1959). Injuries are amongst the leading causes of death, which is a major health concern (Norman et al., 2007). The homicide rate in SA has been shown to be six times higher than any other region worldwide, with the Western Cape having the highest provincial homicide rate (Garrib et al., 2011, Prinsloo et al., 2016). In Western Cape, in 2016, 1 in 16 deaths were due to homicide (7%), this was followed by transport-related deaths (1 in 33 deaths, 3%), suicides accounted for 1.5% (1 in 66 deaths) and other accidents accounted for 2% (1 in 50 deaths).

In instances where the cause of death cannot be determined at autopsy alone, ancillary investigations may be performed. These may include, but are not limited to, toxicology, microbiology, virology, histology, and molecular genetics. Forensic toxicology involves the detection, identification, and/or quantitation of drugs and chemicals in biological specimens, as well as the interpretation and reporting of the said analytical results in the context of medico-legal investigations (Dinis-Oliveira et al., 2010). In post-mortem cases, many biological specimens become available for toxicological analysis that may allow for investigation into the history of drug exposure. Blood and urine are the most commonly used biological specimens to test for the presence of drugs (Saito et al., 2011) and usually allow for interpretation of an acute impairment. While the analysis of these matrices is well established and understood, they have limited drug detection windows and they are prone to being affected by post-mortem artifacts (especially blood) and may be difficult to collect in some cases (e.g., infants, charred or exsanguinated bodies and in decomposition).

In the past years, interest has increased in the use of hair for detection of substances of abuse (Pragst & Balikova, 2006). Compared to blood and urine, which have a limited detection window (usually hours to a few days but can depend on the drug), hair has a longer detection window that can last up to months or several years (Vogliardi et al., 2015). Hence, hair samples

are of interest when investigating an individual's long-term drug exposure (Skopp, 2004; Vogliardi et al., 2015).

Long-term use of drugs has been linked with various physiological changes that might cause an individual to be violent towards themselves or another person (McGregor et al., 2008). Furthermore, studies have demonstrated a relationship between violent deaths and the presence of drugs within the individual (Goldstein, 1985; Marshall et al., 2008; Lundholm et al., 2013). However, this has not been investigated within a local context in South Africa. Thus, the aim of this literature review was to provide an overview of injury mortality in South Africa, the use of hair as a specimen in toxicological analysis and investigating effects of long-term drug exposure particularly within the context of violence. This literature review will focus on the most commonly used drugs used in SA, the most common forms of violence in SA, the possible links between drugs and violent-related fatalities and how hair can be used as an investigative tool in forensic toxicology to detect the long-term use of drugs.

1.2. VIOLENCE-RELATED INJURY AND DEATH RATES

Violence can be defined as the deliberate use of power to intentionally harm oneself or another individual, and can be classified into categories of self-directed, interpersonal, and collective violence (Butchart & Mikton, 2014). The first category of self-directed violence occurs when an individual inflicts harm upon themselves. This includes suicidal thoughts, parasuicide and suicidal acts (WHO, 2014). The second, interpersonal violence, is often directed against a child, intimate partner or elderly member, either by an acquaintance or stranger. This includes acts of homicide and sexual assault (WHO, 2014). Lastly, collective violence is committed by larger groups of individuals, such as organised political groups or terrorist organisations (WHO, 2014). Often, these acts may result in or are a result of war, genocide or gang warfare (Krug et al., 2002).

It is estimated that approximately five million people die as a result of injuries annually, worldwide (WHO, 2010; Nicol et al., 2014). While countries are affected to different degrees; violence-related injuries continue to be amongst the leading causes of death worldwide (Butchart & Mikton, 2014). Violent acts that cause or contribute to death can be intentional, as with homicide and suicide; or unintentional, as is the case in some accidental road traffic

incidents, drowning, burns, suffocation, and poisoning cases (Ballesteros et al.,2018). The World Health Organisation reported that the leading global cause of unnatural death resulting from injury was road traffic incidents, followed by homicide and suicide (WHO, 2010).

The circumstances surrounding these deaths have been shown to vary depending on the age, sex and geographic location of the victims (Butchart & Mikton, 2014). More specifically, males are more frequently the victims of violent deaths than their female counterparts (Garrib et al., 2011). Furthermore, violent deaths are more common in individuals of 15 – 29 years of age (Butchart & Mikton, 2014). Lastly, it has been reported that higher violent death rates are typically seen in low- and middle-income countries, such as SA (Schuurman et al., 2015).

SA has a very high injury mortality rate, falling amongst the countries with the highest homicide rates worldwide (Garrib et al., 2011; Matzopoulos & Myers, 2014). The province of the Western Cape has been reported to be one of the provinces with a high burden of injury in SA (Evans et al., 2018). Between the years of 2011 to 2015, Cape Town, the capital of the Western Cape, was reported to have the highest rate of violent crime when compared to other cities in the Western Cape (City of Cape Town, 2016). Efforts have been made in previous years by the Western Cape provincial Department of Health with the initiation of the Burden of Disease Reduction Project in hopes to alleviate the battle with violence–related injuries and deaths (Matzopoulos & Myers, 2014). However, these efforts have not significantly reduced the homicide rate; the number of lives lost through violence-related injuries remains high in Cape Town.

There has been reported associations between substance abuse and violence (Lundholm et al., 2013). However, it is also understood that there are a number of risk factors that contribute to this complex relationship. However, the association between violent deaths and drug abuse has not been demonstrated or well established in a South African context.

1.3. ACUTE BEHAVIOURAL EFFECTS OF DRUG ABUSE

A vast array of effects may be seen with the administration of various drugs. The resultant effects are dependent on both individual factors and the drug properties. Individual factors include, among others, the sex, physiology and history of drug use (tolerance) of an individual (Boles & Miotto, 2003). Furthermore, different classes of drugs have different pharmacological

properties; in terms of pharmacokinetics and pharmacodynamics, mechanism of action and dependence. Thus, the response to the administration of a drug depends on the type of drug being administered, the dose and the route of administration. A psychoactive drug, also known as a psychotropic substance, is a chemical substance that acts on the central nervous system (CNS), consequently affecting brain function and altering behaviour, consciousness and mood (Whalen, 2015). Many studies have linked violent behaviour with the abuse of certain drugs, affecting both victims and perpetrators (Boles & Miotto, 2003; Marshall et al., 2008; Kuhns & Clodfelter, 2009). The link between violence and drug abuse is complex, as many variables may be involved. Many studies have hypothesised to account for the potential high prevalence of drugs in cases of violent incidents. However, the relationship between the drug administration and violence cannot be concluded merely by the detection of the presence of a drug (Boles & Miotto, 2003).

Goldstein's (1985) tripartite framework is one of the key models describing the aetiology of violence after administering drugs. It proposes three linkages of the associated between drug use and violent behaviour. One being psychopharmacological violence where violent behaviour is due to pharmacological effects of the drug consumed; the second being economic violence in which one commits violence to obtain money to support drug use and lastly, systemic violence between drug dealers and/or drug users.

Boles & Miotto (2003) proposed that neurobiological factors such as monoamine transmitters (serotonin, dopamine and norepinephrine) play a role in violence and substance abuse. Low levels of serotonin have been associated with aggression and psychological disorders (Boles & Miotto, 2003). Alcohol causes the release of serotonin; thus, in cases of alcoholism where there is decreased serotonergic activity due to desensitisation, individuals may become more aggressive and violent (Higley & Linnoila, 2002). Substances such as cocaine, amphetamines and alcohol may increase the release of norepinephrine and dopamine, leading to depletion of the neurotransmitters (Boles & Miotto, 2003). Both norepinephrine and dopamine moderate human aggressive behaviour as they are both involved in behavioural regulation. The excess release and depletion from these substances has been associated with changes in mood and may lead to aggressive behaviour (Boles & Miotto, 2003).

Drug abuse as a social issue is rising in Africa and the trend of use is also changing (INCB, 2015). Most drug users begin with alcohol and cigarettes and end up using other substances of

abuse (Odejide, 2006). Licit drugs that are commonly abused in Africa include alcohol, nicotine (cigarette) and volatile substances (found in glue, shoe polish and petrol). Other psychoactive scheduled drugs that are abused include hypno-sedatives and other prescription drugs such as benzodiazepines and opioids (Odejide, 2006). Illicit drugs that are abused include cannabis (dagga), amphetamine-type stimulants, such as methamphetamine (locally known as "tik"), 3,4-methylenedioxy-methamphetamine (MDMA), opium and its derivatives e.g., heroin, cocaine and crack, methcathinone and methaqualone (which together with diphenhydramine is known as Mandrax) (Odejide, 2006). In SA, most common recreationally used drugs include alcohol, cocaine, methamphetamine, madrax, heroin, and inhalants, sedatives, hallucinogens and opiates and prescription/over-the-counter drugs (Ramlagan et al., 2010; Peltzer, 2010; Peltzer and Phaswana-Mafuya, 2018).

1.4. THE LONG-TERM EFFECTS OF DRUG USE ON VIOLENT BEHAVIOR AND VIOLENT DEATHS

There is currently limited data concerning the behavioural and psychological effects of long-term use of drug use as it pertains to the outcome and circumstances of violent death cases (McGregor et al., 2008). The majority of studies in the literature had focused on the neurological complications associated with long term drug use. It is well established in the literature that long-term drug exposure results in physiological changes in the brain, which in turn, is linked to a chronic altered behavioural state. However, very little is known about the association of this altered behavioural state and the risk of violence and violent deaths (Nieman, 2000). Chronic use of drugs can also be characterised by dependence or addiction. Drug dependence causes a lowered sense of self-control and may lead to erratic and uncontrolled behaviour (Ersche et al., 2012). Drug addiction is a neurological disorder characterised by the continued use of drugs despite it being detrimental to an individual's physical, social or personal well-being (Camí & Farré, 2003). A study by Ersche et al. (2012) found that addiction to stimulant drugs caused abnormalities in the frontostriatal brain systems, which mediate the motor, limbic, cognitive and behaviour functions (Alexander et al., 1986). As a result, these individuals exhibited impairment in behavioural control, decision making, working memory and organisation (Ersche et al., 2012).

To better understand the effect of long-term drug use on behaviour, it is important to understand

the mechanisms of action of various drugs. Both the central and peripheral nervous systems may be adversely affected as a result of excessive drug intake. Changes to the nervous system manifests in dysfunctional neurons, neurotransmitters and neural circuits (Brady, 2012). Most of the commonly abused drugs result in the release of dopamine, a neurotransmitter, which produces euphoria (Blum et al., 2012). However, various drugs may affect the neurotransmitter levels differently, resulting in various behavioural outcomes (Brady, 2012).

Certain drugs result in the depletion of dopamine and may cause feelings of depression, resulting in acts of self-inflicted violence or harm. In other instances, long-term use of MDMA adversely affects the serotonin levels, which results in symptoms of memory impairment, sleep disorders and white matter damage (American Psychiatric Association, 2013). According to Urban et al. (2012) MDMA use, even at a moderate amount, may negatively affect mood, cognition and impulse control. On the other hand, long-term alcohol consumption, depletes both dopamine and serotonin levels, which may increase the risk of aggression (Badaway et al., 2003). In this instance, as with many drugs, the behavioural effects seen as a result of drug use have been associated with factors such as genetic predisposition, high levels of serum testosterone, low levels of serum cholesterol and low levels of serotonin levels (Berman et al., 1997; Badaway et al., 2003).

The long-term use of amphetamine has been linked with the depletion of neurotransmitters (Nieman, 2000). Methamphetamine leads to the release of dopamine, norepinephrine and serotonin. Long-term use of methamphetamine has been demonstrated to damage the white matter, which may increase the chances of a person experiencing hallucinations, anxiety, paranoia and depression (Moeller et al., 2016). The long-term abuse of methamphetamine has also been associated with emotional impulsivity lability, confusion, paranoia and hallucinations (Romenelli & Smith, 2006). Inhalant solvents such as toluene may lead to alterations of dopamine secretion, causing permanent neurological complications, such as peripheral neuropathy and cerebral atrophy (Farre, 1989).

It is evident from the demonstrated effects of both acute and chronic drug misuse that there are severe consequences to drug abuse. Moreover, it has been hypothesised there is a relationship between drug abuse and violence, as evident through Goldberg's tripartite framework. This is of concern, particularly in developing countries such as SA, which experience high rates of drug abuse and violence. In cases of unnatural injury-related death, it is possible to gain some understanding of the role of drugs in death through post-mortem toxicological investigations.

This provides a more acute picture of drug exposure. Currently, there has not been an investigation into the use of alternative specimens, such as hair, in cases of violent death, so as to assess a longer pattern of drug exposure.

1.5. POST-MORTEM FORENSIC TOXICOLOGY

According to South African legislation, all unnatural deaths must undergo a medico-legal autopsy by an authorised medical practitioner within Forensic Pathology Service to determine the cause of death (Inquests Act, 1959). According to the Human Tissues Act (Act 65 of 1983), human tissue can be retained from the deceased for forensic or ancillary investigations into cause of death. In addition, the Acts mentioned permit the pathologist to collect specimens to determine the cause of death. In cases involving forensic toxicological ancillary analyses, femoral blood, vitreous humor, bile, gastric contents and urine are collected for toxicological screening at the National Department of Health, Forensic Chemistry Laboratories. Drugs are typically absorbed into the blood stream via their site of administration and distributed throughout the body by the blood circulation (Whalen, 2015; Liu et al., 2015). Most drugs are metabolised in the liver into hydrophilic metabolites and majority of these metabolites are excreted by means of urination (Moffat et al., 2004). Hence, blood and urine are more routinely collected as they provide a more acute and sub-acute picture of exposure to drugs or chemicals within the deceased. (Skopp, 2004; Liu et al., 2015).

While the analysis of blood and urine matrices is well established and routinely performed, there are limitations associated with each. In addition; the dose, frequency of use, route of administration, type of drug and type of interpretation are factors to consider when selecting a specimen for sampling and interpreting the results obtained from that analysis. In cases where the drugs and/or metabolites cannot be detected in blood and urine or these matrices are not available, alternative specimens may be used (Skopp, 2004). For instance, there is a limited drug detection window and it can often be challenging to preserve these specimens. Therefore, in recent years, there has been a growing interest in the use of alternative specimens for the detection of drugs and metabolites, such as hair or other keratinised structures (Pragst & Balikova, 2006). For instance, keratinised tissue such as hair, nails and the horny layer of the skin are known to retain some drugs and heavy metals (Skopp, 2004). Unlike blood and urine, which have a limited detection window, various drugs and heavy metals can be detected in hair

for up to several years (Skopp, 2004; Vogliardi et al., 2015). These tissues, therefore, are a good source of information for investigating an individual's historic pattern of drug exposure.

The value of hair as a specimen for post-mortem toxicological analyses lies in the ability to determine chronic drug use and possible drug tolerance. This information may be relevant in investigating hair as a specimen for analysis given that there is a framework surrounding drug abuse and violence – however, far more research into all confounding variables is required – but it is a start. In addition, the collection procedure for the specimen is easy and non-invasive, it is difficult to tamper with and depending on the length of it can provide a historical pattern of drug exposure following chronic or single exposure (Kintz, 2017). The amount of drug and/or metabolite incorporated into hair depends on various factors including biological factors (hair structure and pigmentation), individual factors (ethnicity, hair treatment, drug use and disease state) as well as environmental factors and methodological factors (e.g., sampling) (Skopp, 2004). The analytical techniques used to analyse hair have advanced, becoming more robust and sensitive and thereby expanding its potential applications (Kintz, 2004).

1.6. STRUCTURE AND FUNCTION OF HAIR

Hair is a unique structure that is found only on mammals (Buffoli et al., 2014). The main function of hair is to regulate temperature and to protect the skin from mechanical insults (Buffoli et al., 2014). Hair consists of two different components: the hair shaft and the follicle (Kelly et al., 2000). Internally, hair grows into the dermis and subcutaneous fat, which in turn grows at the base into hair bulb, which surrounds the dermal papilla (Randall and Botchkareva, 2003). Hair is constantly produced from the hair bulb of the follicle. The dermal papilla cells, follicular dermal papilla, mucopolysaccharide-rich-stroma, single capillary loop and nerve fibres are enclosed in the hair bulb (Peus and Pittelkow, 1996).

The external section of hair, commonly known as the shaft, is made of up three parts: the cuticle, cortex and medulla. The cuticle is the visible part of the hair shaft and is fully comprised of keratinized cells. This provides a protective layering to the hair, which is necessary since the cuticle is vulnerable to damage by exposure to environmental factors, such as heat or chemicals. Just below the outer cuticle, lies the cortex, which is made up of cortical cells and melanocytes. Lastly, the innermost portion of the hair shaft, the medulla, comprises of spongy keratin and air space. The medulla is not always present in human hair (Cooper, 2011).

Melanin is a skin pigment found in many parts of the body including hair. It is synthesised by melanosomes, which are found in melanocytes (Ings et al., 1984). Eumelanin, pheomelanin, and their oxidative products (oxymeumelanin and oxypheomelanin) determine the colour of hair (Prota, 2000). Eumelanin is mostly found in black and brown hair, oxymeumelanin is found in lighter shades of hair. Pheomelanin causes hair to be red and oxypheomelanin results in a lighter shade of red (Cooper, 2015). The skin of mammals is almost completely covered in hair to facilitate the regulation of body temperature and the evaporation of precipitate from the body. Additionally, hair plays a role in the sensory function of the skin, thereby protecting against minor injury (O'Rahilly et al., 2008). In humans, there are three broad categorisations for hair type. The first, vellus hair, is categorised as short, fine hairs that lack pigmentation. These hairs are commonly contained in the skin covering the majority of the body, including areas such as the eyelids and forehead. In contrast, terminal hairs are long, broad and pigmented. These hairs are typically only found on the scalp, pubic areas, and facial hair (including the eyelashes, eyebrows and beard). Lastly, intermediate hairs share certain characteristics of both vellus and terminal hairs and are typically found on the extremities of adults. The main difference between these three hair types is the way in which they are produced. Unlike vellus hairs, which is not affected by hormonal changes, terminal and intermediate hair is produced in response to hormonal stimuli brought on by puberty. Hence, hair growth in the pubic areas and axilla only start growing during puberty.

Hair growth is a cyclic process, alternating between stages of growth and dormancy (Kintz, 2004). The phases of hair growth are known as the anagen, catagen and telogen phases.The anagen phase is the active growth phase and can last up to a couple of years About 85 % of the hair is in the anagen phase (Kintz, 2004). During the catagen phase, cell division ceases, the hair shaft becomes entirely keratinised and the bulb starts to degenerate (Randall and Botchkareva, 2003). The duration of the catagen phase is dependent on the hair type (Cooper, 2015). During the telogen phase, also known as the resting phase, there is no hair growth. About 10 % to 15 % of the hair is in the telogen phase, depending on one's age. In this stage, hair can easily be removed, hence this stage is usually referred to as shedding (Cooper, 2015). In addition to age, other factors may affect the duration of these phases, including pregnancy, diet, nutrition and genetic disorders (Peus and Pittelkow, 1996).

The average rate for hair growth varies depending on the type of hair. Head hair, typically grows at a rate of 0.60-3.36 cm per month (Harkey, 1993), pubic hair grows at a rate of 0.60-

0.90 cm per month (Pragst et al., 1998), underarm hair at 0.87-1.00 cm per month (Pragst et al., 1998), beard at 1.2 cm per month (Hartwig et al., 2003) and body hair at 0.66-0.96 cm per month (Hartwig et al., 2003). For forensic toxicological analyses, the back-part head also referred to as the posterior vertex region is preferred over other types of hair because it has less variation in growth rate compared to other types of hair (Kintz, 2017). Furthermore, this hair has greater number of actively growing follicles i.e., in the anagen phase (Kintz, 2017).

1.7. INCORPORATION OF DRUGS INTO HAIR

Drugs are incorporated into the hair by simple passive transfer (Usman et al., 2019). It has been proposed that during metabolic activity within the anagen phase, trace elements and drugs or metabolites circulating in the body are incorporated into hair (Kintz, 2007). The detection of drugs in hair is determined by the concentration of the drug in blood and varies amongst drugs because hair has different affinities and binding capacities for various drugs (Kikura et al., 1997; Usman et al., 2019). The rate of transport from blood circulation into hair is mostly determined by lipid solubility of the drug (Cooper, 2015). This is because the melanocytes or keratinocytes found in hair are acidic (pH 3 – 6), thus the transition from plasma (pH 7.3) into hair provides an advantage for basic drugs (Cooper, 2015).

The melanin found in hair facilitates the binding of certain drugs to hair. This has been attributed to melanin being acidic, causing basic drugs to bind with high affinity by protonation (Rothe et al., 1997). Hence, basic drugs are more readily incorporated into hair with a higher melanin content (Uematsu et al., 1989). A study conducted by Rothe et al. (1997) that looked at 15 grey-haired patients, found that there were different concentrations of drugs between white fibres and pigmented fibres, with pigmented ones retaining a higher concentration overall. Henderson et al. (1998) evaluated whether ethnicity plays a factor in the incorporation of drugs in hair and found a 2.7-fold greater concentration of isotopically labelled cocaine in hair of non-caucasians as compared to caucasian volunteers, despite having received the same dose. Rollins et al. (2003) investigated whether codeine was incorporated differently between black, brown, blond and red hair. They found that individuals with black hair had the highest concentration of codeine, followed by brown, blond hair, and red hair (Rollins et al., 2003). Therefore, it is of utmost importance to take into consideration the hair colour and texture as also suggested by the Society of Hair Testing (SoHT) guidelines for drug testing when interpreting the results of toxicological analyses on hair samples (Cooper et al., 2012).

Hair analysis is often used in forensic investigations such as chronic poisoning, child abuse and drug facilitated sexual assault cases (Kintz, 2004). It has also been suggested that hair can be used alongside other traditional specimens to help identify the circumstances surrounding death (Paterson et al., 2009). The interpretation of toxicological results of hair analysis is however, complicated by the possibility of external contamination, which may produce false positives (Kintz, 2012). Furthermore, contamination from external exposure to smoke or from aqueous matrices such as blood, sweat and putrefaction fluid are difficult to remove (Kintz, 2012). There are measures that can be followed to minimise the possibility of false results. However, there is no clear consensus on the most definite decontamination technique (Mantinieks et al., 2018).

The collection procedure for hair is fairly simple. Hair is collected from the posterior vortex (the back of the head), where there is minor variation in growth rate (Cooper et al., 2012). According to these guidelines, samples should be wrapped in aluminium foil and stored in an envelope at room temperature (Paterson et al., 2009). Freezing the samples is not recommended as it may cause hair to swell, leading to the diffusion and/or loss of drugs (Barbosa et al., 2013).

A typical hair sample preparation procedure includes segmentation, decontamination, cutting or pulverisation and extraction (Vogliardi et al., 2015). Segmentation analysis is performed when investigating historical drug exposure, i.e., to determine a pattern of long-term drug exposure (Henderson, 1993). Paterson et al. (2009) reported on cases for which hair was analysed in addition to routine post-mortem specimens to demonstrate history of drug use, abstinence from drug use, lack of tolerance, and compliance to medication in post-mortem cases. During segmentation, hair is cut into 1-cm to 3-cm long sections, which correlates with a period of growth of 1-month (Usman et al., 2019). Hairs are then decontaminated to limit false-positive results and background noise caused by external contamination. Decontamination entails several washing steps with various solvents (Vogliardi et al., 2015). SoHT recommends that both aqueous solutions (methanol: water) should be used in the decontamination step (Copper et al., 2012). However, studies have demonstrated that when an aqueous solution is used in the decontamination step, certain analytes of interest that were extracted may be lost (Johnston, 2015; Pragst & Balikova, 2006). The wash solution should be analysed to limit the possibility of any false-positive results due to external contamination (Pragst & Balikova, 2006). Detection of metabolites in the wash solution may suggest that the washing solvents have extracted analytes. Despite multiple washes, some studies have demonstrated that decontamination does not entirely remove external contamination even when

adhering to SoHT (Mantinieks et al., 2018; Mantinieks et al., 2019). Decontamination is followed by extraction, which is performed to remove the drugs/metabolites from the hair to allow detection and identification. The solvents used during extraction depend on the purpose of the analysis and the analytes of interest. Pulverisation of hair has been recommended, as it causes disintegration of cortical fibres, resulting in maximum extraction of analytes (Miyaguchi et al., 2007). The extract may then be analysed using immunoassay, chromatography and mass chromatography techniques.

1.8. RATIONALE

SA is one of many countries with a high rate of violence-related deaths (Garrib et al., 2011). The Western Cape has a high rate of violence-related fatalities in SA (Matzopoulos & Myers, 2014). This is accompanied by drug abuse that remains prominent in the Western Cape, especially in the form of methamphetamine, Mandrax, cannabis and heroin (van Herdeen et al., 2009; Dada et al., 2018). This increase of both violent deaths and the use of substances of abuse poses a problem as these two are linked. In SA, the use of toxicological analysis in unnatural deaths is still a developing field. Toxicological analysis of blood, urine and other biological samples of individuals who died from violence-related fatalities may assist in investigating the circumstances that led to death.

Toxicological analysis of hair is common in forensic toxicology as hair has a long detection window. A long detection window can help to differentiate between cases of chronic and acute drug abuse especially in cases of long post-mortem interval since hair does not decompose. Drug addiction and long-term use may alter one's behaviour, this is believed to be caused by the physiological changes that drugs cause on the brain (McGregor et al., 2008; Ersche et al., 2012). Chronic use of drugs has been linked with increased depression, aggressive behaviour and social isolation (Homer et al., 2008).

To our knowledge there are no studies in South Africa that have investigated long-term use of drugs in victims of violent death using toxicological analyses. Therefore, it is important to investigate the long-term use of drugs to further understand the role of drugs in violence and fatalities in Cape Town.

A pilot study conducted by Auckloo & Davies (2019) demonstrated a high prevalence of drug detection in a cohort of violent death cases admitted to Salt River mortuary in Cape Town,

Western Cape. In the study, blood, urine and vitreous humour sampled were collected and screened in 104 cases. While hair samples were collected from the same group (in 92 cases), these were not tested during the original study. With the growing interest in the use of hair specimens for toxicological investigations and with the improvement in technologies available for these analyses, it would be beneficial to direct research into analysing these hair samples for long-term drug exposure.

1.9. AIM AND OBJECTIVES

The aim of this study was to investigate the presence of drugs in the hair of a group of 104 victims of violent fatalities in Cape Town, in order to determine a proof of concept of the use of hair in toxicological investigations.

The study objectives were to:

i. Describe the basic characteristics (hair colour and hair length) of the hair samples collected.
ii. Perform qualitative toxicological analyses for the presence of drugs in hair using high-resolution liquid chromatography – quadrupole time-of-flight (UPLC-QTOF) mass spectrometry analysis.
iii. Compare the presence or absence of drugs in various segments of hair samples.
iv. Compare the analytical results of the proposed study to those obtained in the pilot study.

CHAPTER 2: METHODS

2.1. STUDY DESIGN AND SETTING

This was a cross-sectional, descriptive study. Hair samples that were previously collected by Auckloo and Davies (2019) in a pilot study that was conducted in 2015 were analysed. The pilot study was conducted between August and October 2015. It involved a random group of individuals admitted to Salt River mortuary, who had died in relation to an injury, whether accidental, homicidal, or suicidal, and for which the next-of-kin had consented for samples being collected for toxicological screening (which is not routinely performed in these cases). All suspected unnatural deaths in the West Metropole of Cape Town are admitted to Salt River mortuary for an autopsy and medico-legal investigation.

2.2. SAMPLE COLLECTION

A pilot study was previously conducted by Auckloo and Davies (2019) in which hair samples (together with other biological samples) were collected from 104 violent death cases at Salt River Mortuary. These cases included individuals over 10 years of age at death, where the manner of death was reported to be suspected homicide, non-overdose suicide or accidental deaths. All skeletonised remains, decomposed bodies, burn or fire-related deaths or in which hospital survival was more than 24 hours were excluded from this study. Lastly, all cases reported to have fatal drug overdoses, substance or chemical toxicity, gassings as well as road traffic accidents were excluded from the pilot study. Blood, urine, bile and vitreous humor were collected and analysed within the pilot study. Hair samples were only available in 92 cases for inclusion in this study. The cohort consisted of 104 cases in which 94 were homicides (90.4%), 5 were suicides (4.8%), and 5 were accidents (4.8%), deaths were mostly attributed to gunshot wounds (n = 46; 44.2%), stabbings (n = 28; 26.9%), or assaults (n = 18; 17.3%). Less frequent causes of death were hanging (n = 5; 4.8%), drowning (n = 3; 2.9%), multiple causes (e.g., assault-stab, shot-stab cases) (n = 2; 1.9%), electrocution (n = 1; <1%), and ‘freak accidents’ (n = 1; <1%) – events occurring under highly unusual and unlikely circumstances. The overall mean age of victims was 31 years (SD=12), ranging between 10-75 years. There were 99 males

and 5 females. A total of 69 individuals were Black African, 5 were White and a total of 30 individuals were Coloured. Coloured group in Southern Africa is a multiracial group who have an ancestry from a various population (Posel, 2001). A pencil-sized section of hair was removed from the back of the head (posterior vertex) of each individual using scissors. The hair was collected without roots, three cases had dreadlocks, one was braided with artificial hair, three had dyed hair and one case had dried blood. The hair was placed in a piece of white paper, folded in the form of a pharmacist's or druggist's fold, and stored in a tamper-proof evidence bag at room temperature under dry conditions until further analysis.

2.2. ETHICS

This research was approved by the University of Cape Town (UCT) Human Research Ethics Committee (pilot study: HREC Ref. 324/2015; current study HREC Ref: 369/2019) (Appendix A). Informed consent was obtained in the original pilot study from the next-of-kin for analysis of all the specimens. No contact was made with next-of-kin in this study and results were not provided to them. This study made use of the anonymised unique research numbering of the original study, thus rendering data confidential and anonymous in this study.

2.3. ASSESSING HAIR COLOUR AND LENGTH

The colour and length of the hair for each sample was determined as suggested in the SoHT guidelines (Cooper et al., 2012). Both hair colour and hair length were analysed by the principal investigator. Hair length was measured using a standard ruler. The hair length was determined using a ruler and recorded in millimetres. Hair colour was assessed by proxy using the Munsell soil-colour chart (2009) (Munsell Color, 2009). It is recognised that the use of this chart serves other purposes, but the goal was to provide an objective means of determining the hair colour through standardisation. It is also recognised however that there is a subjective factor still involved in determination of these colours, which is a limitation. The variation between hair colour, texture, and possible treatments could not be further assessed given the random sample collection and lack of control samples.

2.4. TOXICOLOGICAL SCREENING

2.4.1. CHEMICALS AND REAGENTS

Internal standards doxepin d-$_3$ and diazepam d-$_5$ in methanol (10 ug/ml) were purchased from Restek (Restek Corporation, Bellefonte, PA, USA). Dichloromethane (DCM) and hydrochloric acid (HCl) (5.0 N) were purchased from ThermoFisher (Kandel, GmbH, Germany). Pure ethyl alcohol was purchased from Sigma-Aldrich (Darmstadt, Germany). Methanol (Methanol 215) was obtained from Romil Pure Chemistry (Romil Limited, Waterbeach, CAM, UK). All chemicals were of high-performance liquid chromatography (HPLC) grade or better. Ultrapure water was obtained with a Milli-Q® Water Purification System (Merck, Burlington, MA, USA).

2.4.2. SAMPLE PROCESSING

Hair samples were prepared according to the method previously developed by Johnston (2015; 47-48), this method was used as a basis for the sample preparation. Three washing procedures were analysed in Johnston (2015) study, ethanol and DCM procedure gave the best result. Hair samples that were more than 100m were segmented by trimming two sections of 1-cm each from the distal and proximal ends of the hairs. All segments were subsequently analysed. The segmented hair samples were used to assess the historical pattern of drug use.

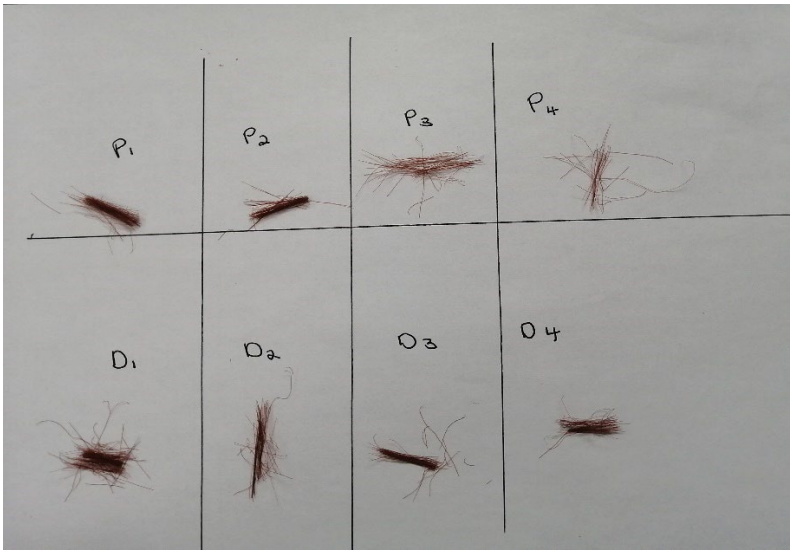

Figure 1. Trimming and segmentation of hair (P1,P2,P3,P4 and D1, D2, D3,D4)

2.4.2.1. DECONTAMINATION OF HAIR

A total mass of 20 mg of hair was weighed out and was then placed in Omni Bead Ruptor Bead Mill tube (Omni, Inc., Kennesaw GA, USA). The samples were decontaminated with 1 ml ethanol (100%). The samples were mixed for 10 seconds using a vortex and left for 10 minutes at room temperature. The ethanol was then removed and discarded. A second and third decontamination step was performed in the same way but this time with 1 ml DCM. The final washing of DCM was transferred to a 1.5 mL micro-centrifuge tube for further analysis. Only the final wash was analysed. The hair samples were washed to limit false-positive results and background noise that might be caused by external contamination.

2.4.2.1. EXTRACTION OF HAIR

An extraction solution of methanol and aqueous HCl (1 M) in a 4:1 ratio was made up. Four metal beads were added to the bead mill homogenizer tubes along with 1 mL of the extraction solution and the internal standard (160 ng/mL). The tubes were allowed to stand at room temperature for 10 minutes. The samples were pulverised using an Omni Bead Mill Homogenizer (Omni International, Gerogia, USA) at 5.5 m/s for 1 minute with a dwell time of 30 seconds for 5 cycles. The samples were centrifuged at 4000 rpm for 5 minutes. The supernatant (800 µl) was transferred to a microcentrifuge tube. Both the transferred supernatant and last wash of dichloromethane were centrifuged at 13000 rpm for 5 minutes. A 700 µL aliquot of each supernatant was transferred into separate borosilicate tubes. The samples were concentrated by evaporation using the miVac Modular Concentrator Series (SP Scientific, Warminster, USA). The miVac Modular Concentrator Series was run at a temperature of 500C, run time of 1 hour 15 minutes (heat time was 15 minutes and total time was 1 hour), the concentrator was run at auto mode and the method was water. Following evaporation, the samples were reconstituted with 150 µl deionised water. All 150 µL from both tubes was transferred into a glass insert placed within 2 mL screw neck HPLC glass vials. A total of 32 cases weighed more than 5 mg, making the samples eligible for analysis. Only 5 mg (12 cases) or 10 mg (20 cases) was measured out and the sample preparation method was adjusted using appropriate ratios of reduced solvent volumes for the rest of the method.

2.4.3. TOXICOLOGICAL ANALYSIS

The hair samples were screened for the presence of common toxicological substances using the SCIEX X500R LC-QTOF (Framingham, MA, USA). Sample acquisition was performed using the SCIEX Method™ Application for Forensics Toxicology used by the Division of Clinical Pharmacology at UCT (Fu et al., 2017).

An ExionLC™ AC HPLC system was used to obtain chromatographic separation, which was performed using the Phenomenex Kinetex® phenyl-hexyl column (2.6 µm x 50 x 4.6 mm). A gradient method using mobile phase A (MPA) (10 mM ammonium formate in water) and mobile phase B (MPB) (0.05 % formic acid in methanol) was run at 600 µL/min. Mass spectrometric (MS) analysis was performed in positive ionisation mode and MS and MS/MS data were collected using SWATH® acquisition. The SCIEX OS Software (AB Sciex PTY. Ltd., Woodlands, Singapore) was used to acquire, analyse, and process the data.

Due to time-constraints, hair could not be collected for a control group, therefore all samples were assessed along with negative urine controls were used.

2.5. DATA ANALYSIS

The SCIEX OS Software was used to perform data processing, which was set up previously within the Division of Clinical Pharmacology. Findings were considered positive if the mass error, retention time, isotope ratio difference and library score were within required ranges. Data processing was performed in house by the LCQTOF technician within the Clinical Pharmacology Laboratory. Drugs were considered present when both ISTDs (doxepin $d\text{-}_3$ and diazepam $d\text{-}_5$ in methanol (10 ug/ml)) were detected and when the variables indicated above passed. Basic descriptive statistics were performed on the analysed data.

CHAPTER 3: RESULTS

3.1. CHARACTERISTICS OF SAMPLES

Samples available from the original study included 92 (88 %) hair samples of 104 cases. Two cases were excluded from this cohort as the sample weight was too low (0.4 mg and 1.2 mg) and thus only 90 hair sample were included for analysis. The mean weight of these 90 samples was 16.9 mg (SD=5.4 mg). Most hair samples (87.8%, n= 79) were 'black' in colour (Figure 1). The colours 'black and reddish black', 'light grey' and 'reddish brown and strong brown' hair categories had the least number of samples, with only one hair sample each. Five hair samples were 'black and white' and three were 'greyish brown and yellow'. Hair colour was assessed by proxy using the Munsell soil-colour chart (2009) (Munsell Color, 2009). The colours 'black and reddish black', 'reddish brown and strong brown', 'black and white' and 'greyish brown and yellow' mean that the hair sample had two colours.

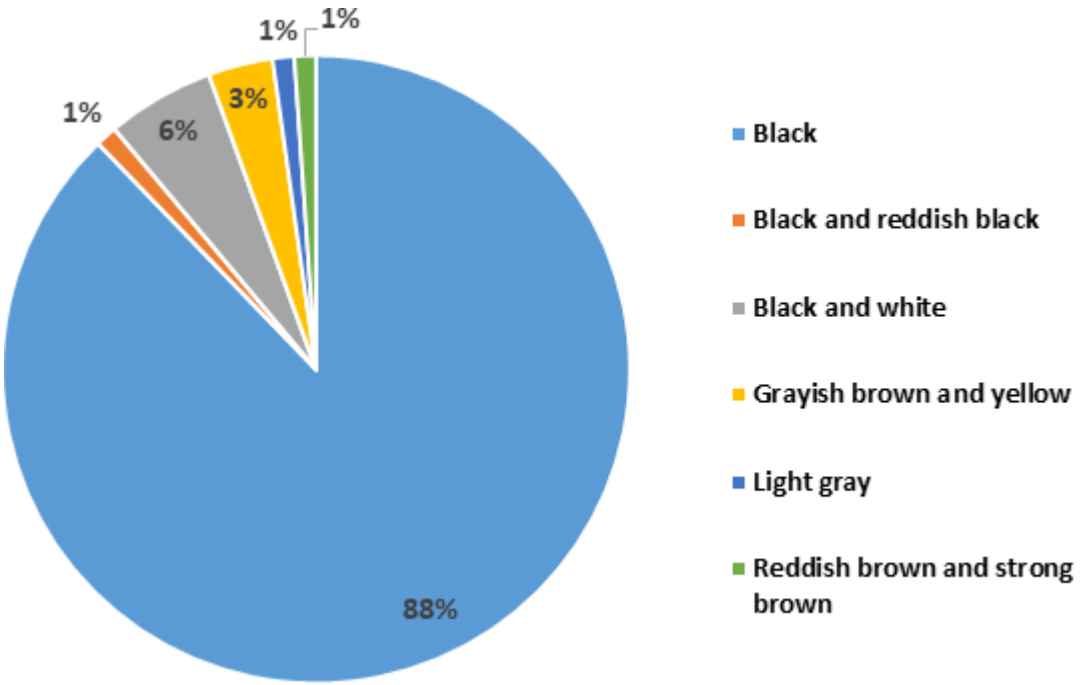

Figure 2: Classification of hair colour for samples collected from violent death cases at Salt River mortuary for toxicological analysis. n=90.

The cohort consisted of 90 cases with an overall mean age of victims was 30 years (SD=11.8); ranging between 10-75 years. There were 85 males and 5 females (Table 1). About two-thirds of the victims

were Black Africans (57 (63.3%)), one-third were mixed-race (29 (32.2%)) and 4 (4.4%) were Caucasian. Suspected homicides accounted for 81 (90 %) cases, and 5 cases were reported as accidents (4.4%). Of the 81 homicides, 79 (97.5%) were male, and 2 (2.5%) were female victims. Black Africans accounted for 53 (58.9%) of these homicides, mixed-race for 27 (30%) of homicide cases and Whites for 1 (1.1%) homicide. Suicide accounted for 5 (4.8%) cases. Of the overall suicide cases, 3 (60%) were males and 2 (40%) were females. The rest were accidents (n=4, 4.4%), 3 (75%) were males and 1 (25%) was female.

Table 1 Cohort (n=90) demographic and apparent manner of death data

Victim Characteristics		**Homicide**	**Suicide**	**Accident**	**Total**
		Total number of cases [n (%)]			
Sex	Male	79 (97.5%)	3 (60%)	3(75%)	85 (94.4%)
	Female	2 (2.5%)	2 (40%)	1 (25%)	5 (5.6%)
Ethnicity	Black African	53 (58.9%)	2 (40%	2 (50%)	57 (63.3%)
	Coloured	27 (30%)	1 (20%)	1 (25%)	29 (32.2%)
	White	1 (1.1%)	2 (40%)	1 (25%)	4 (4.4%)

The apparent causes of death for these 90 cases were assault (16.7%, n=15), stabbings (28.9%, n=26), drownings (3.3%, n=3), electrocution (1.1%, n=1), hanging (5.6%, n=5), gunshot injuries (43.3%, n=39) and combination of injuries (1.1%, n=1). There were no females who died from stabbing, electrocution, or assault. One female died from drowning, two from hanging and two died from gunshot injuries. Males accounted for the rest of the apparent causes of death. The most prominent apparent cause of death in Black Africans was stabbings (n=23), followed by gunshot injuries (n=17), assault (n=12), hanging (n=2), drowning, electrocution and assault and stabbing all had one case each. In Coloureds, the most prominent apparent cause of death was from gunshot injuries (n=21), followed by assault and stabbing which had 3 cases each and lastly hanging and drowning which had one case each. With Whites, the most prominent cause of death was hanging (n=2), followed by gunshot injuries, and drowning with one case each.

3.2. PRESENCE OF TOXICOLOGICAL SUBSTANCES

Using LC-QTOF different drugs and/or metabolites were detected, these were considered present (positive) when both ISTDs were detected and when the data met acceptable criteria (Figure 2). Drugs and/or metabolites were detected in 74 cases (82.2%), from these cases a total of 54 different drugs and/or metabolites were detected.

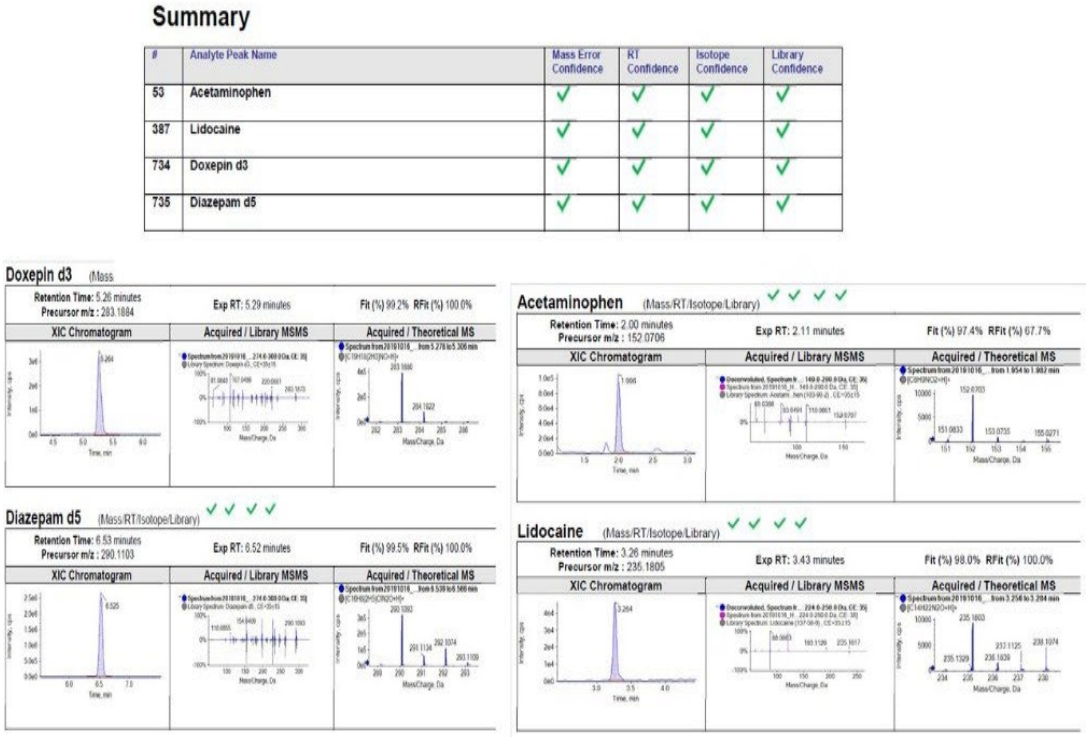

Figure 3 LC-QTOF chromatogram showing ISTDs (doxepin-d3 and diazepam-d5) and substances (acetaminophen and lidocaine) detected

These drugs and/or metabolites were categorised according to their drug class previously described (Auckloo & Davies, 2019) (Table 3). This included: amphetamines, tricyclic antidepressants, dissociative anaesthetic, opiates and opioids, OTC, prescription, and other drugs, benzodiazepines / z-drugs, cocaine, sedative-hypnotics, and antihistamines. Acetaminophen (over-the-counter non-steroidal anti- inflammatory drug) was detected in 47% of the cases and was the most prominent substance detected. This was followed by caffeine (18.9%) (a stimulant) and diphenhydramine (antihistamine) (12%). Methamphetamine was the most commonly detected illicit substance.

Table 2 List of substances detected categorised according to their drug class

Class of substances	**Substance(s) detected**
Amphetamines	Methamphetamine, Methcathinone, Amphetamine[†]
Tricyclic antidepressants	Amitriptyline, Maprotiline, Nortriptyline*, Protriptyline
Dissociative anaesthetic	Ketamine

Opiates and Opioids	3-monoacetylmorphine[+], Codeine, Dextromethorphan, Dextrorphan/Levorphanol, Hydromorphone, Hydrocodone, Morphine, Naloxone, O-Desmethyltramadol[***], Tramadol
OTC, Prescription, and Other Drugs	Acetaminophen, Benzocaine, Caffeine, Carbamazepine, Carbendazim, Chlorazanil, Climbazole, Ciprofloxacin, Mirtazapine, Methcathinone, Noscapine, Papaverine, Pentoxyverine, Phenytoin, Quinine, Ritalinic acid, Salicylamide, Sildenafil, Sulfadoxine, Sulfamethoxazole, Sulfathiazole, Thiabendazole, Verapamil
Benzodiazepines / Z-drugs	Diazepam, Zolpidem, Zopiclone
Cocaine	Benzoylecgonine[++], Ecgonine methyl ester[++], Cocaine, Cocaethylene[+++]
Sedative-hypnotics	Methaqualone
Antihistamines	Chlorpheniramine, Cinnarizine, Diphenhydramine**, Doxylamine, Orphenadrine, Pyrilamine

[†] Metabolite of methamphetamine
[+]Metabolite of heroin
[++]Metabolite of cocaine
[+++]Formed when cocaine and ethanol coexist
*Nortriptyline is a metabolite of amitriptyline
**Diphenhydramine is usually detected with methaqualone due to Mandrax use
[***] O-Desmethyltramadol is metabolite of tramadol

3.2.1. COMPARATIVE TOXICOLOGY

When comparing suspected manner of death, detection of drugs and/or metabolites was frequent in homicide cases (85% cases positive), followed by accidents (75%) and suicides (60%). In accident cases (n=4), no drugs were detected (n=1), acetaminophen was detected (n=1), illicit drugs such as amphetamine, methamphetamine, methaqualone and thiabendazole were detected (all in one case) and in the last case acetaminophen, amitriptyline, tramadol were detected.

In apparent suicide cases (n=5), there were no drugs and/or metabolites detected in two cases. In the other three cases, tricyclic antidepressants and opiates and opioids were detected. Amitriptyline, maprotiline and cocaine were detected in one case; acetaminophen, amitriptyline, chlorpheniramine, maprotiline, nortriptyline, protriptyline and tramadol in another case; on the last case acetaminophen, caffeine, chlorpheniramine, cinnarizine, doxylamine and tramadol were detected.

Out of the 81 homicide cases, drugs and/or metabolites were detected in 66 (81.5%) cases. These included amphetamines, tricyclic antidepressants, dissociative anaesthetic, opiates and opioids, OTC, prescription, and other drugs, benzodiazepines / z-drugs, cocaine, sedative-hypnotics and antihistamines.

Out of 104 cases in Auckloo and Davies (2019) study, drugs and/metabolites were detected in 63 (61%) cases (where femoral blood, heart blood, urine, vitreous humor, and bile were tested). From these cases, a total of 43 different analytes and/or metabolites were detected, 16 of which were detected in this study. These drugs were benzodiazepines (diazepam, zolpidem and zopiclone), amphetamines (methamphetamine, amphetamine and methcathinone), cocaine, opiates/opioids (codeine, hydromorphine and tramadol), methaqualone, hallucinogens (ketamine), antidepressants (protriptyline and nortriptyline), OTC/prescription drugs (lidocaine and acetaminophen).

Twenty-two (22) drugs and/or metabolites were not detected in this current study (but were detected in the Auckloo and Davies (2019) study), were benzodiazepines (nordiazepam, temazepam and midazolam), amphetamines (MDMA), opiates/opioids (morphine 3-β-d-glucuronide, morphine, fentanyl and norfentanyl), hallucinogens (PCP), antidepressants (norfluoxetine, fluoxetine, desipramine and clomipramine), OTC/Prescription drugs (diltiazem, metformin, amlodipine, atenolol, dexamethasone, ranitine, reserpine, salicylamine and sibutramine).

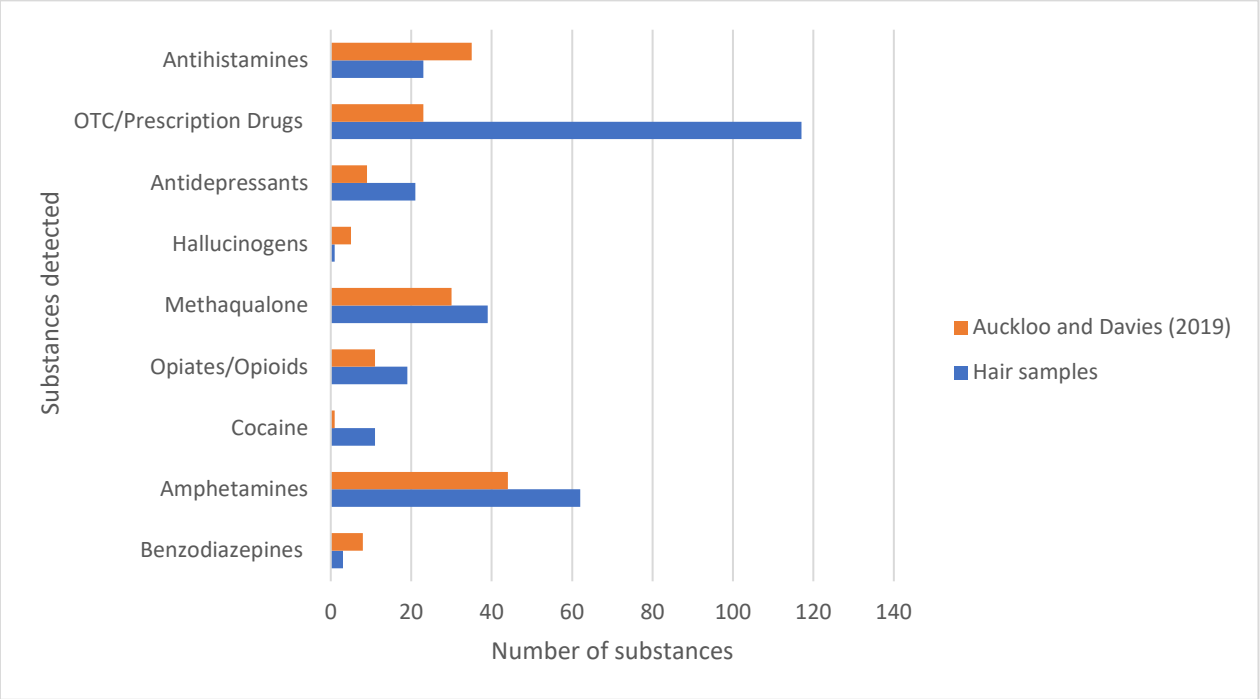

Figure 4 Comparative analysis of the number of drugs and/or metabolites categorised according to their drug classes detected between both studies (current study and Auckloo and Davies (2019)).

3.2.2. DETECTION OF MULTIPLE SUBSTANCES

Drugs and/or metabolites were detected in 75 cases, with a combination of different drugs and/or metabolites detected in 60 (67%) cases (Table 3). In homicide cases, multiple drugs and/or metabolites were detected in 55 cases. In suicide and accident cases, multiple drugs and/or metabolites were detected in 3 and 2 of those cases, respectively (Table 3). Acetaminophen, methamphetamine, methaqualone, and diphenhydramine were the most prominent combination in homicide cases.

Table 3 Cases in which multiple drugs were detected in hair samples from N=90 overall cases

ID#	Suspected Manner of Death	Cases with multiple drugs detected (n=60)
1	Accident	Acetaminophen, Amitriptyline, Maprotiline, O-Desmethyltramadol, Pentoxyverine, Protriptyline, Tramadol, Zopiclone
2	Accident	Amphetamine, Methamphetamine, Methaqualone, Thiabendazole
3	Homicide	Methaqualone, Verapamil
4	Homicide	Acetaminophen, Methaqualone, Caffeine, Climbazole, Methamphetamine, Verapamil
5	Homicide	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone, Naloxone, Salicylamide
6	Homicide	Acetaminophen, Caffeine, Dextromethorphan, Hydromorphone, Sulfadoxine
7	Homicide	Amphetamine, Methamphetamine, Methaqualone, Orphenadrine
8	Homicide	Acetaminophen, Amphetamine, Caffeine, Dextromethorphan, Diphenhydramine, Methamphetamine, Sulfamethoxazole, Sulfathiazole
9	Homicide	Amphetamine, Methamphetamine, Methaqualone, Diphenhydramine, Tramadol
10	Homicide	Acetaminophen, Amitriptyline, Chlorpheniramine, Ciprofloxacin, Dextromethorphan, Dextrorphan/Levorphanol, Maprotiline, Nortriptyline, Protriptyline, Quinine, Benzocaine, Codeine, Doxylamine, Hydrocodone, Ciprofloxacin, Methamphetamine, methaqualone
11	Homicide	Acetaminophen, Amphetamine, Caffeine, Chlorpheniramine, Methamphetamine, O-desmethyltramadol, Tramadol, Verapamil, Thiabendazole,
12	Homicide	Acetaminophen, Diphenhydramine, Lidocaine, Methaqualone
13	Homicide	Caffeine, Methamphetamine, Verapamil
14	Homicide	Acetaminophen, Caffeine
15	Homicide	Acetaminophen, Amphetamine, Diphenhydramine, Maprotiline, Methamphetamine, Nortriptyline, Protriptyline
16	Homicide	Amitriptyline, Caffeine, Maprotiline, Methamphetamine, Methaqualone
17	Homicide	Acetaminophen, Caffeine, Diphenhydramine, Methamphetamine, Methaqualone
18	Homicide	Amphetamine, Caffeine, Methamphetamine, Tramadol, Verapamil
19	Homicide	Acetaminophen, Methaqualone
20	Homicide	Acetaminophen, Lidocaine
21	Homicide	Chlorazanil, Lidocaine, Methamphetamine
22	Homicide	Acetaminophen, Methamphetamine, Methaqualone
23	Homicide	Methamphetamine, Methaqualone
24	Homicide	6-Monoacetylmorphine, Acetaminophen, Diphenhydramine, Methamphetamine, Methaqualone
25	Homicide	Caffeine, Cocaethylene, Cocaine, Ketamine, Lidocaine, Ritalinic acid
26	Homicide	Acetaminophen, Diazepam, Methamphetamine, Methaqualone
27	Homicide	Methamphetamine, Methaqualone, Quinine
28	Homicide	Methamphetamine, Methaqualone
29	Homicide	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone, Thiabendazole, Amitriptyline
30	Homicide	Acetaminophen, Methamphetamine, Methaqualone
31	Homicide	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone
32	Homicide	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone
33	Homicide	Acetaminophen, Carbendazim, Methamphetamine, Quinine
34	Homicide	Amphetamine, Methamphetamine, Methaqualone, Methcathinone

35	Homicide	Benzoylecgonine, Cocaethylene, Cocaine, Ecgonine methyl ester, Acetaminophen, Chlorazanil, Pyrilamine, Tramadol, Lidocaine, Thiabendazole
36	Homicide	Diphenhydramine, Methamphetamine, Methaqualone
37	Homicide	Sulfamethoxazole, Verapamil
38	Homicide	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone, Thiabendazole
39	Homicide	Acetaminophen, Hydromorphone, Lidocaine, Methamphetamine, Methaqualone, Noscapine, Papaverine, Phenytoin
40	Homicide	Acetaminophen, Methamphetamine, Methaqualone
41	Homicide	Methaqualone, Thiabendazole
42	Homicide	Acetaminophen, Codeine, Hydrocodone, Methamphetamine, Methaqualone, Thiabendazole
43	Homicide	Acetaminophen, Amphetamine, Diphenhydramine, Lidocaine, Methamphetamine, Methaqualone, Thiabendazole
44	Homicide	Acetaminophen, Quinine, Verapamil
45	Homicide	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone
46	Homicide	Acetaminophen, Amphetamine, Diphenhydramine, Lidocaine, Methamphetamine, Methaqualone, Midazolam, O-Desmethyltramadol, Tramadol
47	Homicide	Acetaminophen, Methamphetamine, Methaqualone
48	Homicide	Acetaminophen, Amphetamine, Diazepam, Diphenhydramine, Methamphetamine, Methaqualone
49	Homicide	Methamphetamine, Quinine, Verapamil
50	Homicide	Methamphetamine, Methaqualone, Acetaminophen, Quinine
51	Homicide	Amphetamine, Methamphetamine
52	Homicide	Acetaminophen, Amphetamine, Diphenhydramine, Methamphetamine, Thiabendazole
53	Homicide	Caffeine, Sildenafil
54	Homicide	Acetaminophen, Amphetamine, Caffeine, Methamphetamine, Verapamil
55	Homicide	Acetaminophen, Caffeine, Diphenhydramine, Methaqualone, Sildenafil
56	Homicide	Acetaminophen, Amphetamine, Diphenhydramine, Hydrocodone, Lidocaine, Maprotiline, Methamphetamine, Methaqualone, Nortriptyline, Protriptyline
57	Homicide	Caffeine, Ketamine
58	Suicide	Amitriptyline, Maprotiline, Cocaine, Doxylamine, Mirtazapine, Zolpidem
59	Suicide	Acetaminophen, Amitriptyline, Chlorpheniramine, Maprotiline, Nortriptyline, Protriptyline, Tramadol
60	Suicide	Acetaminophen, Caffeine, Chlorpheniramine, Cinnarizine, Doxylamine, Tramadol, Codeine, Pyrilamine, Thiabendazole, Cocaine

3.2.3. HISTORICAL PATTERN OF DRUG USE

Eleven hair samples were more than 100 mm in length and were segmented by trimming two sections of 1 cm each from the distal and proximal ends of the hairs. The overall mean length of these samples was 37.9 mm. Drugs and/or metabolites were detected in all the eleven cases and multiple substances were detected in each case (Table 6). However, in some cases there were no drugs and/or metabolites detected in some segments. In case TXH003, there were no drugs and/or metabolites detected in one segment (P1), however, drugs and/or metabolites were detected in the other three segments (P2, D1, D3). In case TXH042, there were no drugs and/or metabolites

detected in three segments (P1, P2, D2), however, in one segment (D1), amphetamine, caffeine, methamphetamine, tramadol and verapamil were detected.

In 9 cases, where drugs and/or metabolites were detected in all segments, some drugs and/or metabolites were detected in all four segments (P1, P2, D1, D3). In case TXH012, amitriptyline, maprotiline, cocaine was detected in most segments. In case TXH024, acetaminophen, amitriptyline, chlorpheniramine, ciprofloxacin, dextromethorphan, dextrorphan/levorphanol, maprotiline, nortriptyline, protriptyline and quinine were detected in three segments. In case TXH030, acetaminophen, amphetamine, methamphetamine and tramadol were present in all segments. In sample TXH045, acetaminophen, amitriptyline, maprotiline and tramadol were detected in all the segments. In sample TXH059, acetaminophen, amphetamine, methamphetamine, methaqualone and thiabendazole were detected in all segments except for segment D2. In case TXH066, acetaminophen was the only drug that was detected in all the segments. In case TXH071, benzoylecgonine and cocaine were detected in all segments. In case TXH092, methamphetamine and methaqualone were detected in all the segments except for one (D1). In sample TXH101, acetaminophen, diphenhydramine and methaqualone were detected in all segments.

Table 4 Drug detection in proximal and distal hair segments for assessment of the historical pattern of drug use. n = 11. P1: first segment cut from the proximal end; P2: second segment cut from the proximal end; D1: first segment cut from the distal end; D2: second segment cut from the distal end.

Sample ID	Segment	Drug detected
TXH003	P1	None
	P2	Acetaminophen, Methaqualone
	D1	Caffeine, Climbazole, Methamphetamine
	D2	Verapamil
TXH012	P1	Amitriptyline, Maprotiline, Cocaine
	P2	Amitriptyline, Maprotiline, Cocaine
	D1	Doxylamine, Mirtazapine, Zolpidem
	D2	Amitriptyline, Cocaine
TXH024	P1	Acetaminophen, Amitriptyline, Chlorpheniramine, Ciprofloxacin, Dextromethorphan, Dextrorphan/Levorphanol, Maprotiline, Nortriptyline, Protriptyline, Quinine
	P2	Acetaminophen, Amitriptyline, Benzocaine, Chlorpheniramine, Codeine, Dextromethorphan, Dextrorphan/Levorphanol, Doxylamine, Hydrocodone, Maprotiline, Nortriptyline, Protriptyline, Quinine
	D1	Acetaminophen, Amitriptyline, Chlorpheniramine, Ciprofloxacin, Methamphetamine, methaqualone
	D2	Amitriptyline, Cocaine, Maprotiline
TXH030	P1	Acetaminophen, Amphetamine, Caffeine, Chlorpheniramine, Methamphetamine, O-desmethyltramadol, Tramadol

	P2	Amphetamine, Chlorpheniramine, Methamphetamine, Tramadol, Verapamil
	D1	Acetaminophen, Amphetamine, Methamphetamine, Thiabendazole, Tramadol
	D2	Acetaminophen, Amphetamine, Methamphetamine, Thiabendazole, Tramadol
TXH042	P1	None
	P2	None
	D1	Amphetamine, Caffeine, Methamphetamine, Tramadol, Verapamil
	D2	None
TXH045	P1	Acetaminophen, Amitriptyline, Maprotiline, O-Desmethyltramadol, Pentoxyverine, Protriptyline, Tramadol, Zopiclone
	P2	Acetaminophen, Amitriptyline, Maprotiline, O-Desmethyltramadol, Pentoxyverine, Protriptyline, Tramadol, Zopiclone
	D1	Acetaminophen, Amitriptyline, Maprotiline, O-Desmethyltramadol, Pentoxyverine, Protriptyline, Tramadol, Zopiclone
	D2	Acetaminophen, Amitriptyline, Maprotiline, Pentoxyverine, Protriptyline, Tramadol
TXH059	P1	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone, Thiabendazole
	P2	Acetaminophen, Amphetamine, Methaqualone, Thiabendazole
	D1	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone, Thiabendazole
	D2	Acetaminophen, Amitriptyline, Methaqualone
TXH066	P1	Acetaminophen, Caffeine, Chlorpheniramine, Cinnarizine, Doxylamine, Tramadol
	P2	Acetaminophen, Codeine, Doxylamine, Pyrilamine, Tramadol
	D1	Acetaminophen, Pyrilamine
	D2	Cocaine, Thiabendazole
TXH071	P1	Benzoylecgonine, Cocaethylene, Cocaine, Ecgonine methyl ester
	P2	Acetaminophen, Chlorazanil, Pyrilamine, Tramadol
	D1	Benzoylecgonine, Cocaine, Lidocaine
	D2	Benzoylecgonine, Cocaine, Lidocaine, Thiabendazole
TXH092	**P1**	Methamphetamine, Methaqualone, Quinine
	P2	Acetaminophen, Methaqualone
	D1	Acetaminophen
	D2	Methamphetamine, Methaqualone
TXH101	**P1**	Acetaminophen, Caffeine, Diphenhydramine, Methaqualone
	P2	Acetaminophen, Caffeine, Methaqualone, Sildenafil
	D1	Benzocaine, Carbamazepine, Diphenhydramine, Methaqualone,
	D2	Acetaminophen, Methamphetamine, Methaqualone

CHAPTER 4: DISCUSSION AND CONCLUSION

SA is one of many countries that is faced with the issue of increased drug use and violence, which in turn has contributed to the increased injury mortality rate in the country (Seedat et al., 2009). This study looked at the preliminary drug detection in a group of hair samples obtained from a random deceased cohort who died in violent circumstances in Cape Town, SA. Various studies have demonstrated the association of drugs and violent related deaths (Marshall et al, 2008; Kuhns & Clodfelter, 2009; Lundholm et al., 2013). Furthermore, Auckloo and Davies (2009) conducted a preliminary investigation into drugs detected in a group of violent death cases. The goal was to demonstrate the concept that drugs are detected and should be investigated further in violent deaths. Auckloo and Davies (2009) study was in agreement with previous literature as different drug and/or metabolites were detected in 60% of the victims of violent death. Auckloo and Davies (2009) performed toxicological analyses in blood, urine, vitreous humour and bile. Even though hair samples were collected, the hair was not analysed. In this study the hair was analysed, and the study demonstrated that hair can be used as a supplementary sample during toxicological investigation as various drugs and/or metabolites were detected in the hair. It can also be used to investigate long-term exposure in SAPS cases as segmentation of hair demonstrated that different drugs may be detected in different hair segments. Furthermore, more than half of the cases demonstrated poly-use of drugs, this was also the case in the Auckloo and Davies (2009) study.

4.1 CHARACTERISTIC OF SAMPLES

SA is one of many countries with a very diverse population. There are approximately 58 775 022 people in SA, 47 443 259 (80,7%) are Black Africans, 5 176 750 (8.8%) are Coloureds, 4 652 006 (7.9%) are Whites and 1 503 007 (2.6%) are Asian/Indians (Stats SA, 2019). Cape Town is estimated to have a population of 4 004 793 with 41,6% being Black Africans, 33.6% being Coloureds, 21,8% Whites, Asians 1.3% and other (unspecified) being 1.7% (City of Cape Town, 2016). In the current study about 63% were Black Africans, 32% were Coloureds and 4% were Whites. It should be noted that this ethnic profile of this cohort does not represent the general ethnic profile of the population of Cape Town.

With this diverse ethnicity, hair colour ought to be one of the variables when performing hair analysis. The SoHT guidelines state that it is mandatory to record hair colour when conducting hair analysis (Cooper et al., 2012). This is because people with more melanin have been demonstrated to incorporate more drugs into hair compared to people with less melanin (Kronstrand et al, 1999). This was observed in a study by Kronstrand et al., (2001), where individuals who had light blonde hair had low concentration amphetamine compared to individuals with darker hair. Most of the hair samples in this study were black (88%, n=79). However, hair colour was not taken into consideration when interpreting the results. Hair colour and texture are mostly important when conducting quantitative toxicological studies. To our knowledge, there are currently no studies in South Africa that have taken hair colour into consideration when interpreting toxicological hair results. This poses as a gap in toxicological hair analysis and warrants further investigation.

In the current study, the total number of included violent fatalities was 90. Males accounted for about 94% of the cohort while females accounted for only about 6% of the cohort. This is in accordance with previous literature that has demonstrated that males are more likely to be prevalent in violent deaths compared to females (Garrib et al., 2011; Butchart & Mikton, 2014). The main manner of deaths were homicides, suicides and accidents. The main apparent causes of the death were from gunshot injuries, stabbings, assault, drowning, hanging, electrocution and stabbing and assault. Homicides attributed for 90% of the cases, in which females accounted for only 2.5% of these homicides and males for 97.5%.

In 2016, studies indicated that SA has one of the highest homicide rates in the world (World Health Organization, 2018). It was indicated by an incidence of 33.1 per 100 000 population inclusive of both sexes, when compared to other African countries such as Angola which only reported an incidence of 9.8 per 100 000 population (World Health Organization, 2018). A study by Prinsloo et al. (2016) found that the Western Cape had the highest rates of homicides in the country and in 2009 the province ranked fourth in the number of homicides committed out of the nine provinces in SA. The apparent cause of death in these homicide cases was mostly from gunshot injuries which accounted for 39 (48.1%), this was followed by stabbings 27 (33.3%), assault with 14 (17.2%) cases and lastly stabbings and assault accounted for one case. There were no females who died from stabbing and assault, and only two females died from shooting. This study is in accordance with previous literature that has demonstrated that there is a significant difference between males and females in homicide cases (Lindegaard, 2017). The overall mean age of the current study was 30 years (SD=11.8). Studies have shown that

victims in their twenties and thirties are more prone to be a victim of homicide (CSVR, 2008). However, it is important to take into consideration that this study only evaluated a small group of violent fatalities and thus limit the extent of further discussion. A larger sample and further investigation would be needed to further explain the high dominance of males in homicide cases.

Hair analysis may provide many advantages to both SAPS and forensic pathologists when trying to solve cases. Unlike other samples (blood and urine) it is unlikely to tamper with hair, you can determine a person's historical drug use, it is possible to collect an identical sample, or more accurately, a sample representing the same time window, it is not possible to 'beat the test' in hair analysis, meaning that drug abstinence a few days before sample collection will not remove drugs from the hair

4.2. QUALITATIVE TOXICOLOGICAL ANALYSIS

It was found that drugs and/or metabolites were detected in 83% of the cases (n=75), in which 54 different drugs and/or metabolites were detected. Acetaminophen was the most prominent licit substance (47%) detected, followed by caffeine (18.9%) and diphenhydramine (12%). Acetaminophen commonly known as paracetamol is a medication commonly used to treat fever and pain (Gazarain et al., 2014). The high detection of paracetamol is expected as most individuals take paracetamol for treatment of pain and fever. Another substance expected to be highly detected is caffeine, as caffeine is a natural stimulant that is found in tea, coffee and cocoa. Diphenhydramine is an antihistamine used to relieve the effects of histamine such as allergy, fever and itchiness. Diphenhydramine - which was one of the most detected drugs - is usually added to drugs such as methaqualone to enhance effects.

Methamphetamine was the most detected illicit substance (54%, n=49) followed by methaqualone (n=39,43%). Mandrax commonly known as methaqualone is a highly addictive potent quinazolines, it is similar to glutethimide and barbiturates both in structure and function (McCarthy et al., 2003). It was first prescribed for sleeping disorders, anxiety and high blood pressure. However, it was banned due to its side effects especially when smoked with cannabis (McCarthy et al., 2003). While methaqualone has been banned, it continues to be a major problem in SA (McCarthy et al., 2003). The effect of oral administration of methaqualone is quite different from smoked methaqualone. The effect of smoked methaqualone is quicker to

onset and lasts longer, While the acute effects are slower in oral administration and have a shorter duration (McCarthy et al., 2003). Banned Mandrax combined methaqualone base (250 mg), and diphenhydramine hydrochloride (25 mg) (UNODC. 2014). The high detection of diphenhydramine and methaqualone individually may be because of this banned combination to make Mandrax and people wanting enhanced effects of the drug combination. However, there might be other underlying reasons for the detection of methaqualone-diphenhydramine in this cohort and a larger sample would be needed to make any correlation.

In the Western Cape, and specifically Cape Town, the most common illicit drug of abuse is methamphetamine (Dada et al., 2016). Methamphetamine also known as tik in SA is highly addictive and is commonly abused for its effects, which include a feeling of euphoria and increased energy (Panenka et al., 2013). Worldwide, approximately 35 million people use methamphetamine (Romanelli & Smith, 2006), making it the second most used illicit drug (Watt et al., 2014). Several factors increase the high usage of methamphetamine, one being that it is highly addictive and the other one is the fact that it is cheap and easily manufactured (Romanelli & Smith, 2006). The use of methamphetamine has been associated with aggressive behaviour and interpersonal violence (Darke et al., 2008; Watt et al., 2014), it has also been associated with depression, which contributes to suicide rates (Darke et al., 2008). Studies have shown individuals commit violent acts while under the influence of methamphetamine (Sommers et al., 2006; Romanelli & Smith, 2006). This violent behaviour after using methamphetamine has been associated with paranoia, psychosis, hallucinations and depression (Sommers et al., 2006; Darke et al., 2008). Methamphetamine was the most prominent illicit drug in both the current study and the pilot study. Previous epidemiological data of SA has shown that there is a high detection rate of methamphetamine particularly in Cape Town (van Heerden et al., 2009). The high detection rate of methamphetamine in this study is consistent with the epidemiology data.

There were prominent differences in the type of drugs and/or metabolites that were detected across the apparent manner of deaths. In suicide cases, cocaine was the only illicit drug that was detected. Other drugs detected included antidepressants, opiates and antihistamines. Antidepressants are used to treat mental disorders and for the prevention of suicide (Nischal et al., 2012). However, literature has suggested that some antidepressants may worsen suicidal thoughts in vulnerable patients (Methling et al., 2018). In the current study, the association between antidepressants and suicide cannot be inferred as the sample size was not big enough

to make such conclusions. However, this indicates that further studies need to be conducted using hair to investigate long-term use or exposure to antidepressants in suicide cases.

In fatalities due to accidents, drugs were detected in drowning cases (3) and no drugs were detected in the electrocution case. In fatalities from drowning, illicit drugs were only detected in one case. Other drugs detected included antidepressants, opiates and antihistamines. Studies have shown that some drugs that cause psychomotor impairment and affect cognition such as antidepressants have been detected in drownings (Ahlm et al., 2013). In this study there were only three cases where the apparent cause of death was drowning, and drugs and/or metabolites were detected in all these cases. This shows that hair can be used in drowning deaths during toxicological analysis, to determine the drugs or/metabolites a person was exposed to preceding death.

Drugs and/or metabolites were mostly detected in homicide fatalities. Homicide cases attributed for 90% cases of the total cases. Majority of these cases were Black Africans (58.9%), followed by Coloureds (30%) and lastly Whites (1.1%). These high detections of males and Black Africans has been associated with high unemployment rates, poverty, income inequality, access to firearms and drugs (Prinsloo et al., 2016*).* Homicide cases have been associated with drug use. In the current study, drugs were detected in more than 80% of the homicide cases. These included amphetamines, tricyclic antidepressants, dissociative anaesthetic, opiates and opioids, OTC, prescription, and other drugs, benzodiazepines / Z-drugs, cocaine, sedative-hypnotics and antihistamines. Drugs that have sedative effects such benzodiazepines may increase the risk of one being a victim of violence, since these drugs may make a person vulnerable due to intoxication (Darke, 2010). Psychostimulants such as cocaine and methamphetamine have been associated with violence (Darke, 2010). The most detected illicit drugs in these cases were methamphetamine and methaqualone. Methaqualone is one of many drugs that is prominent in homicide cases specifically in SA (WHO, 2016). Due to its effect, methamphetamine is highly detected in violent fatalities.

4.3 HAIR SEGMENTATION

One of the advantages of hair analysis is that it can show an individual's historical drug use. This is possible through segmentation. Segmentation was performed in eleven hair samples. Drugs and/or metabolites were detected in all the eleven cases and multiple substances were detected in each case. The segments were cut in 1 cm which is equivalent to approximately one

month as reported in literature (Cuypers & Flanagan, 2018). However, some individuals may have different hair growth rates. Most cases indicated a historical pattern of drug use as the same drugs were detected in different hair segments (P1, P2, D1, D3), and different body samples (bile, blood, urine and vitreous humor).

Hair segments have been used in different studies to indicate historical drug use or single use (George & Braithwaite, 1997; Clauwaert et al., 2000). In sample TXH042, there were no drugs and/or metabolites detected in three segments (P1, P2, D2), in one segment (D1) amphetamine, caffeine, methamphetamine, tramadol and verapamil were detected. This may indicate single use as none of these drugs were detected in the other samples (bile, blood, urine, and vitreous humor). This detection of drugs in one segment, shows that it possible to detect drugs after single use in South African hair. This may help in drug facilitated sexual assaults, where drugs such gamma-hydroxybutyrate (GHB) are usually used. GHB detection window is short in blood and urine, thus the use of hair may help detect it.

Segmented hair analysis makes it possible to detect changes in drug use over a period. Cases such as TXH030, TXH059, TXH066 and TXH092 indicated historical drug use as the same drugs were detected in different segments and in other samples (blood, urine, bile, and vitreous humor). This indicates that segmented hair samples may be used as supplementary samples to determine the use of drugs in the period (month or longer) before death, and in cases of gestational exposure to identify past maternal drug abuse for example. Hair analysis is not a routine practise in post-mortem toxicology in South Africa, however, it may be of value in cases where the history of drug use of a decedent is in question, or if insight into drug tolerance is needed. This study may form a basis for stronger investigations into hair testing in post-mortem cases in future.

4.4. POLY-DRUG USE

The use of illegal drugs has increased over the past two decades in SA and many individuals use more than one drug (World Drug Report, 2015). A combination of different substances was detected in 60 cases (67%) cases. In the current study the combination of acetaminophen, methamphetamine and methaqualone detected in 30% (n=27), the combination of methamphetamine and methaqualone was detected in 33 (37%) cases. In a study by Dada et al. (2016) patients admitted in treatment centres in the Western Cape, 48% of the patients used multiple substances. With methamphetamine used as a primary substance and methaqualone

and cannabis as secondary (Dada et al., 2016). This is in accordance with both this study and the pilot study (Auckloo and Davies, 2019). Methaqualone, diphenhydramine and cannabis are mostly smoked together. In SA, Mandrax (methaqualone and diphenhydramine) is crushed and mixed with cannabis and then smoked, the combination is called white pipe (Peden et al., 2000). This white pipe mixture has been detected in violence-induced injuries in patients (Foster, 1996). The use of Mandrax has been linked to changes in a person's behaviour such as psychosis, being violent and drug-dependency (Tennant, 1973). Poly-drug use is a problem is SA, especially in violence-induced fatalities. This study demonstrates that hair can be used as a supplementary sample in cases of violence-induced fatalities where poly-drug use is highly likely. The high rate of detected illicit drugs in this study warrants further investigation using more reliable and validated methods, into the role of hair testing as one factor in understanding the contribution of drugs to violence.

4.5. LIMITATIONS

Forensic toxicological hair analysis comes with many challenges and interpretive limitations. The possibility of external contamination is one such limitation. Decontamination procedures are used to remove all external contamination of drugs on the hair. However, it is recognised that decontamination procedures may not be uniform or consistent for all drugs of interest (Mantinieks et al., 2019). In the current study, several wash samples were positive for drugs. This indicates that is possible that the wash process is not yet fully optimised. Further investigation into optimising the washing process may be warranted.

As mentioned in literature, hair colour plays a role in the amount drug that is incorporated into hair. Studies have shown that different amounts of drugs were incorporated in different hair colours, even though known amounts of drug were administered (Henderson, 1998; Stout. 2007). Additionally, the hair products used should be taken into consideration when interpreting hair results, this is because cosmetic treatment of hair such as bleaching, dyeing and perming may alter the results (Skopp et al., 1997). Some studies have shown that opiates, cocaine and benzodiazepines are degraded after bleaching (Yegles et al., 2000; Wennig, 2000). This study did not assess the role of hair colour, texture, or treatment, in the detection of drugs. This requires a controlled study, and it is recommended to select a smaller number of drugs to investigate further (e.g. methamphetamine and methaqualone, which were commonly found in

this study). A quantitative method of analysis would also be required. This would assist further in future interpretation of results.

The method utilised was within an external laboratory. It was not validated fully for the purpose of this analysis as that was not within the realm or scope of this project. It is recognised that this is a limitation, however, the given the method involved a data independent acquisition using SWATH® analysis, it was envisaged that most drugs of interest would be detected, and that these would be within the library of over 700 analytes of forensic interest. It is recommended that validation of techniques for qualitative and quantitative forensic work be pursued in future.

This study was part of a pilot investigation into the role of routine toxicological testing in violent deaths. The hair was collected from a random group of individuals and may not represent detection rates in the larger population of violent fatalities. It is recommended that future studies include controls from deceased individuals who have not died violently to further investigate the value of hair testing in a post-mortem context.

This study could not determine how the individuals were exposed to the drugs (voluntarily or involuntarily) or the specific time frame of use, nor the specific route of administration (inhalation, injection, oral etc.) from these results, especially as no quantitative data was obtained. If quantitative analyses are pursued, they should account for requirements set out by the SoHT. Hair toxicology is still a challenging science that requires further research going forward to make it practically usable in a local context within medico-legal casework.

4.6. CONCLUSION

As mentioned, many studies have been conducted investigating the prevalence of drugs in violent fatalities. However, most of these studies used acute samples to perform toxicological screening. This study was a pilot investigation to look at the comparison of hair toxicological screening to more acute samples. The study showed that more drugs were detected in hair than other samples (blood, bile, vitreous humor and urine). This is consistent with literature as hair has a long detection window. It also shows the relative detection rate of certain substances that warrant further investigation in hair (e.g., methaqualone or methamphetamine). This study shows that hair can be used for toxicological investigation in a South African context, provided controls are put in place, the method is quantitative and contextual information is assessed.

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A.1. ETHICS APPROVAL LETTER

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
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14 June 2019

HREC REF: 369/2019

Ms B Davies
Division of Pathology
Department of Forensic Medicine & Toxicology
Room 5.07 Falmouth Building-FHS

Dear Ms Davies

PROJECT TITLE: POST-MORTEM TOXICOLOGICAL ANALYSIS OF HAIR IN VIOLENT FATALITIES: AN INVESTIGATION INTO LONG-TERM DRUG EXPOSURE (SUB-STUDY LINKED TO 324/2015) MPHIL CANDIDATE - MS P Z MNISI

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 June 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Ms Precious Mnisi will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

pp **PROFESSOR M BLOCKMAN**
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB0000103

NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

A.2. CHARACTERISTICS OF HAIR SAMPLES AND SUBSTANCES DETECTED IN HAIR, FEMORAL BLOOD, HEART BLOOD, URINE, VITREOUS HUMOUR AND BILE.

Table 1 . Table demonstrating variables of hair samples and substances detected in hair, femoral blood, heart blood, urine, vitreous humour and bile. N/A: The sample was not available, -: no substances were detected

CAS NO	Segments	Age	Sex	Ethnicity	Apparent cause of death	Suspected Manner of Death	Weight (mg)	Hair colour	Substance Present in other matrices	Substance Present in hair	Washes	Hair	Femoral Blood	Heart Blood	Urine	Vitreous Humor	Bile
TXH001		26	M	C	Shot	Homicide	10	Black	Y	Y	–	Methaqualone, Verapamil	Diazepam	Nordiazepam	–	–	N/A
TXH002		33	M	C	Shot	Homicide	10	Black	Y	N	–	–	–	N/A	Methamphetamine Amphetamine	–	–
TXH003	P1	43	M	C	Shot	Homicide	20	Black	N	N	–	–	–	–	–	–	N/A
	P2						20			Y	–	Acetaminophen, Methaqualone					
	D1						20			Y	–	Caffeine, Climbazole, Methamphetamine					
	D2						20			Y	–	Verapamil					
TXH004		25	M	A	Stab	Homicide	20	Black	Y	Y	–	Methamphetamine	–	N/A	Diphenhydramine Methamphetamine Amphetamine	–	THC-COOH
TXH005		42	M	A	Shot	Homicide	20	Black and white	Y	Y	–	Acetaminophen	–	N/A	Amphetamine	–	–
TXH006		19	M	A	Stab	Homicide	20	Black	N	N	–	–	–	–	–	–	N/A
TXH008		36	F	A	Shot	Homicide	20	Black	N	N	–	–	–	–	N/A	–	–
TXH009							0.4	Black	Y				–	N/A	Atropine Benzoylecgonine Concaine	–	Benzoylegonine
TXH010		44	M	C	Assault	Homicide	20	Black	N	Y	–	Methaqualone	–	–	–	–	N/A
TXH012	P1	75	F	W	Hang	Suicide	5	Grayish brown and yellow	Y	Y	–	Amitriptyline, Maprotiline, Cocaine	–	–	Desipramine	–	N/A
	P2						5			Y	–	Amitriptyline, Maprotiline, Cocaine					
	D1						5			Y	–	Doxylamine, Mirtazapine, Zolpidem					
	D2						5			Y	–	Amitriptyline, Cocaine					
TXH013		30	M	A	Stab	Homicide	10	Black	Y	Y	–	Acetaminophen	–	–	–	–	N/A
TXH014		28	M	A	Assault	Homicide	5	Black	Y	Y	–	Cocaine	Diphenhdramine Methaqualone	Diphenhydramine	Methamphetamine Amphetamine Diphenhydramine Methaqualone	Methamphetamine Diphenhydramine Methaqualone	N/A
TXH015		20	M	C	Shot	Homicide	20	Black and white	Y	Y	6-monoacetylmorphine, acetaminophe, hydromorphone, methamphetamine, methaqualone, morphine, naloxone	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone, Naloxone, Salicylamide	Methamphetamine Diphenhydramine Methaqualone	Diphenhydramine Methaqualone	Methamphetamine Amphetamine Diphenhydramine Methaqualone	Methaqualone	N/A
TXH016		25	M	A	Stab	Homicide	20	Black	N	N	–	–	–	–	–	–	N/A
TXH017		50	M	C	Stab	Homicide	20	Black	Y	Y	–	Acetaminophen, Caffeine, Dextromethorphan, Hydromorphone, Sulfadoxine	Diphenydramine Methaqualone	Diphenhydramine Methaqualone	Methamphetamine Amphetamine Diphenhydramine Methaqualone Morphine Hydromorphine	Diphenhydramine Methaqualone	N/A
TXH018		28	M	A	Shot	Homicide	5	Black	N	Y	–	Acetaminophen	–	–	–	–	N/A
TXH020		38	M	A	Stab	Homicide	10	Black	N	Y	–	Amphetamine, Methamphetamine, Methaqualone, Orphenadrine	–	N/A	–	–	–
TXH021		26	M	C	Shot	Homicide	20	Black	Y	Y	–	Acetaminophen, Amphetamine, Caffeine, Dextromethorphan, Diphenhydramine, Methamphetamine, Sulfamethoxazole, Sulfathiazole	Methamphetamine Diphenhydramine Methaqualone	Methamphetamine Diphenhydramine Methaqualone	Methamphetamine Amphetamine Diphenhydramine Methaqualone Morphine-3-B-D-glucuronide	–	N/A
TXH022		31	M	A	Stab	Homicide	5	Black	N	Y	–	Cocaine	–	–	–	Sibutramine	N/A
TXH023		17	M	A	Assault	Homicide	5	Black and white	Y	Y	–	Amphetamine, Methamphetamine, Methaqualone, Diphenhydramine, Tramadol	Methamphetamine Diphenhydramine Methaqualone	Methamphetamine Diphenhydramine Methaqualone	Methamphetamine Amphetamine Diphenhydramine Methaqualone Morphine-3-B-D-glucuronide	–	N/A
TXH024	P1	49	F	A	Shot	Homicide	20	Black	Y	Y	–	Acetaminophen, Amitriptyline, Chlorpheniramine, Ciprofloxacin, Dextromethorphan, Dextrorphan/Levorphanol, Maprotiline, Nortriptyline, Protriptyline, Quinine	Metformin	Metformin Amlodine	N/A	–	Metformin Amlodipine
	P2						20			Y	–	Acetaminophen, Amitriptyline, Benzocaine, Chlorpheniramine, Codeine, Dextromethorphan, Dextrorphan/Levorphanol, Doxylamine, Hydrocodone, Maprotiline, Nortriptyline, Protriptyline, Quinine					
	D1						10			Y	–	Acetaminophen, Amitriptyline, Chlorpheniramine, Ciptofoxacin, Methamphetamine, methaqualone					
	D2						10			Y	–	Amitriptyline, Cocaine, Maprotiline					
TXH026		10	M	A	Drown	Accident	20	Black	Y	Y	–	Acetaminophen	–	–	Codeine	–	N/A
TXH028		26	M	A	Shot	Homicide	20	Black	N	N	–	–	–	–	–	–	N/A
TXH029		20	M	A	Stab	Homicide	20	Black	N	Y	–	Acetaminophen, Lidocaine	–	–		–	N/A
TXH030	P1	32	M	C	Shot	Homicide	20	Black and white	Y	Y	–	Acetaminophen, Amphetamine, Caffeine, Chlorpheniramine, Methamphetamine, O-desmethltramadol, Tramadol	Methamphetamine	Methamphetamine	Methamphetamine Amphetamine Diphenhydramine	–	Methamphetamine Diphenhydramine
	P2						20			Y	–	Amphetamine, Chlorpheniramine, Methamphetamine, Tramadol, Verapamil					
	D1						20			Y	–	Acetaminophen, Amphetamine, Methamphetamine, Thiabendazole, Tramadol					
	D2						20			Y	–	Acetaminophen, Amphetamine, Methamphetamine, Thiabendazole, Tramadol					
TXH031		42	M	A	Hang	Suicide	10	Black	Y	N	–	–	–	–	–	–	N/A

CAS NO	Segments	Age	Sex	Ethnicity	Apparent cause of death	Suspected Manner of Death	Weight (mg)	Hair colour	Substance Present in other matrices	Substance Present in hair	Washes	Hair	Femoral Blood	Heart Blood	Urine	Vitreous Humor	Bile
TXH032		20	M	A	Assault	Homicide	20	Black	Y	Y	–	Acetaminophen, Diphenhydramine, Lidocaine, Methaqualone	–	–	Lidocaine	–	N/A
TXH033		20	M	A	Stab	Homicide	5	Black	Y	N	–	–	Katamine	Katamine	Katamine	Katamine	N/A
TXH034		25	M	A	Assault	Homicide	20	Black	Y	Y	–	Caffeine, Methamphetamine, Verapamil	–	–	–	–	N/A
TXH035		25	M	A	Stab	Homicide	20	Black	N	Y	–	Acetaminophen, Caffeine	–	N/A	–	–	–
TXH036		28	M	C	Shot	Homicide	20	Black	Y	Y	–	Acetaminophen, Amphetamine, Dephenhydramine, Maprotiline, Methamphetamine, Nortriptyline, Protriptyline	Methaqualone	Methaqualone	Methaqualone THC-COOH Diohenhydramine	Methaqualone	–
TXH037		60	M	W	Shot	Homicide	20	Light gray	N	N	–	–	–	–	–	–	–
TXH039		49	M	C	Shot	Homicide	10	Black and white	N	Y	–	Amitriptyline, Caffeine, Maprotiline, Methamphetamine, Methaqualone	–	–	–	–	N/A
TXH040		18	M	C	Shot	Homicide	5	Black	Y	Y	–	Acetaminophen, Caffeine, Diphenhyramine, Methamphetamine, Methaqualone	Diphenhydramine Methaqualone Atenolol	Diphenhydramine Methaqualone	Methaqualone Clomipramine	Diphenhydramine Methaqualone	N/A
TXH041		20	M	A	Stab	Homicide	20	Black	Y	N	–	–	THC-OH	THC-OH Lidocaine Norfluoxetine	–	–	N/A
TXH042	P1	20	M	C	Shot	Homicide	20	Reddish brown and strong brown	Y	N	–	–	THC-OH Methaqualone Norfluoxetine	THC-OH Methaqualone Norfluoxetine	Nordiazepam Morphine-3-B-D-glucuronide	Methaqualone	N/A
	P2						20			N	–	–					
	D1						20			Y	–	Amphetamine, Caffeine, Methamphetamine, Tramadol, Verapamil					
	D2						20			N	–	–					
TXH043		27	M	A	Assault	Homicide	20	Black	Y	Y	–	Acetaminophen, Methaqualone	N/A	N/A	MDMA	–	Diphenhydramine Methamphetamine THC-OH Temazepam Diltiazem
TXH044		30	M	A	Assault	Homicide	20	Black	Y	Y	–	Chlorazanil, Lidocaine, Methamphetamine	Diphenhydramine THC-OH Methaqualone Norfluoxetine	THC-OH Methaqualone	Methaqualone Lidocaine	Methaqualone	N/A
TXH045	P1	63	F	W	Drown	Accident	20	Grayish brown and yellow	Y	Y	–	Acetaminophen, Amitriptyline, Maprotiline, O-Desmethyltramadol, Pentoxyverine, Protriptyline, Tramadol, Zopiclone	THC-OH Fluoxetine Protriptyline Nortriptyline	N/A	N/A	Norfluoxine Protriptyline Nortriptyline Tramadol	Nordiazepam THC-OH Diltiazem Protriptyline Nortriptyline Tramadol
	P2						20			Y		Acetaminophen, Amitriptyline, Maprotiline, O-Desmethyltramadol, Pentoxyverine, Protriptyline, Tramadol, Zopiclone					
	D1						10			Y	–	Acetaminophen, Amitriptyline, Maptotiline, O-Desmethyltramadol, Pentoxyverine, Protriptyline, Tramadol, Zopicione					
	D2						10			Y	–	Acetaminophen, Amitriptyline, Maptotiline, Pentoxyverine, Protriptyline, Tramadol					
TXH046		26	M	C	Shot	Homicide	20	Black	Y	Y	Acetaminophen, Methamphetamine, Methaqualone	Acetaminophen, Methamphetamine, Methaqualone	Diphenhydramine THC-OH Methaqualone	Diphenhydramine THC-OH Methaqualone	Diphenhydramine Ranitine Reserpine	–	THC-COOH Nordiazepam
TXH047		35	M	A	Stab	Homicide	10	Black	Y	Y	–	Methamphetamine, Methaqualone	THC-OH	THC-OH	–	–	N/A
TXH048		30	M	C	Shot	Homicide	20	Black	Y	Y	Methamphetamine, Methaqualone	6-Monoacetylmorphine, Acetaminophem, Diphenhydramine, Methamphetamine, Methaqualone	THC-OH Methaqualone	Diphenhydramine THC-OH Methaqualone	Diphenhydramine	Methamphetamine Methaqualone	N/A
TXH049		30	M	A	Stab	Homicide	10	Black	Y	Y	–	Caffeine, Cocoethylene, Cocaine, Ketamine, Lidocaine, Ritalinic acid	Lidocaine	THC-OH Lidocaine Ketamine Norfentanyl	Lidocaine	THC-OH Temazepam Diltiazem	N/A
TXH050		31	M	A	Assault	Homicide	20	Black	Y	Y	Methamphetamine, Methaqualone	Acetaminophen, Diazepam, Methamphetamine, Methaqualone	THC-OH Methaqualone	THC-OH Diphenhydramine	MDMA	Diphenhydramine Methaqualone	N/A
TXH052		21	M	A	Shot	Homicide	10	Black	N	Y	–	Methamphetamine, Methaqualone, Quinine	–	–	–	–	N/A
TXH053		23	M	A	Shot	Homicide	20	Black	Y	Y	–	Acetaminophen	Methaqualone	N/A	N/A	–	–
TXH054		32	M	C	Shot	Homicide	20	Black	Y	Y	–	Methamphetamine, Methaqualone	–	N/A	Diphenhydramine	Diphenhydramine	Diphenhydramine THC-COOH
TXH055		30	M	A	Stab	Homicide	10	Black	N	Y	–	Caffeine	–	–	–	–	N/A
TXH056		28	M	A	Shot	Homicide	10	Black	Y	N	–	–	–	–	Amphetamine Codeine	–	N/A
TXH057		23	M	A	Shot	Homicide	20	Black	N	Y	–	Methaqualone	–	–	N/A	–	–
TXH059	P1	25	M	C	Shot	Homicide	20	Black and reddish black	Y	Y	–	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone, Thiabendazole	Methamphetamine Amphetamine Diphenhydramine Methaqualone	N/A	Diazepam Methamphetamine Amphetamine Diphenhydramine Methaqualone Morphine-3-B-D-glucuronide	Methamphetamine Amphetamine Diphenhydramine Methaqualone	Diazepam Methamphetamine Amphetamine Diphenhydramine Methaqualone Morphine-3-B-D-
	P2						20			Y	–	Acetaminophen, Amphetamine, Methaqualone, Thiabendazole					
	D1						20			Y	–	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone, Thiabendazole					
	D2						20			Y	–	Acetaminophen, Amitriptyline, Methaqualone					
TXH060		41	M	A	Shot	Homicide	10	Black	Y	Y	–	Caffeiine	–	N/A	Amphetamine Codeine	–	N/A
TXH062		17	M	A	Stab	Homicide	20	Black	Y	N	Methaqualone	–	N/A	–	Codeine Phencyclidine Methcathinone	–	Methcathinone
TXH063		31	M	A	Shot	Homicide	20	Black	Y	Y	Methamphetamine, Methaqualone	Acetaminophen, Methamphetamine, Methaqualone	Methamphetamine Amphetamine Diphenhydramine Methaqualone	Methamphetamine Amphetamine Diphenhydramine Methaqualone	N/A	Methamphetamine Amphetamine Diphenhydramine Methaqualone	Methamphetamine Amphetamine Diphenhydramine Methaqualone
TXH064		65	M	C	Hang	Suicide	20	Black	Y	Y	–	Acetaminophen, Amitriptyline, Chlorpheniramine,	Metformin	Metformin	N/A	Metformin	Metformin Amlodipine

CAS NO	Segments	Age	Sex	Ethnicity	Apparent cause of death	Suspected Manner of Death	Weight (mg)	Hair colour	Substance Present in other matrices	Substance Present in hair	Washes	Hair	Femoral Blood	Heart Blood	Urine	Vitreous Humor	Bile
TXH065		16	M	A	Shot	Homicide	20	Black	Y	Y	Methamphetamine, Methaqualone	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone	Diphenhydramine Methaqualone Salicylamine	Diphenhydramine Methaqualone	Diphenhydramine Methaqualone Morphine-3-B-D-glucuronide	Methamphetamine Diphenhydramine Methaqualone	N/A
TXH066	P1	15	F	W	Hang	Suicide	20	Grayish brown and yellow	Y	Y	–	Acetaminophen, Caffeine, Chlorpheniramine, Cinnarizine, Doxylamine, Tramadol	Acetaminophen Codeine	Diphenhydramine Acetaminophen	N/A	Acetaminophen Codeine Doxylamine	Acetaminophen Codeine
	P2						20			Y	–	Acetaminophen, Codeine, Doxylamine, Pyrlamine, Tramadol					
	D1						20			Y	–	Acetaminophen, Pyrlamine					
	D2						20			Y	–	Cocaine, Thiabendazole					
TXH067		34	M	C	Shot	Homicide	20	Black	Y	Y	Methamphetamine, Methaqualone	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone	Methamphetamine Amphetamine Diphenhydramine Methaqualone	Methamphetamine Amphetamine Diphenhydramine Methaqualone	Methamphetamine Amphetamine Diphenhydramine Methaqualone Morphine Morphine-3-B-D-glucuronide	Methamphetamine Amphetamine Diphenhydramine Methaqualone	N/A
TXH068		32	M	A	Stab	Homicide	20	Black	N	N	–	–	–	–	–	–	N/A
TXH069		20	M	A	Shot	Homicide	20	Black	N	Y	–	Acetaminophen, Carbendazim, Methamphetamine, Quinine	–	–	–	–	–
TXH070		21	M	A	Assault	Homicide	20	Black	Y	Y	Methamphetamine, Methaqualone	Amphetamine, Methamphetamine, Methaqualone, Methcathinone	Methamphetamine Amphetamine Diphenhydramine Methaqualone	Methamphetamine Amphetamine Diphenhydramine Methaqualone	Methamphetamine Amphetamine Diphenhydramine Cocaine Methaqualone Mophine-3-B-D-glucuronide	Methamphetamine Amphetamine Diphenhydramine Methaqualone Ranitine	N/A
TXH071	P1	23	M	A	Shot	Homicide	20	Black	N	Y	–	Benzoylecgonine, Cocaethylene, Cocaine, Ecgonine methyl ester	–	–	–	–	N/A
	P2						20			Y	–	Acetaminophen, Chlorazanil, Pyrilamine, Tramadol					
	D1						20			Y	–	Benzoylecgonine, Cocaine, Lidocaine					
	D2						20			Y	–	Benzoylecgonine, Cocaine, Lidocaine, Thiabendazole					
TXH072							1.2	Black	Y				Methamphetamine Diphenhydramine Methaqualone	Diphenhydramine Methaqualone	Methamphetamine Diphenhydramine	Methamphetamine Diphenhydramine Methaqualone	N/A
TXH073		18	M	A	Assault	Homicide	5	Black	Y	Y	–	Diphenhydramine, Methamphetamine, Methaqualone	Methaqualone	Methaqualone	Methamphetamine Diphenhydramine Methaqualone	Diphenhydramine Methaqualone	Methamphetamine Diphenhydramine Methaqualone
TXH075		32	M	A	Stab	Homicide	20	Black	Y	Y	–	Sulfamethoxazole, Verapamil	–	–	Methamphetamine Diphenhydramine	–	N/A
TXH077		21	M	A	Stab	Homicide	20	Black	Y	Y	Methaqualone	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone, Thiabendazole	Methaqualone	Methaqualone	Methamphetamine Diphenhydramine	–	–
TXH078		31	M	A	Stab	Homicide	20	Black	N	Y	–	Acetaminophen, Methaqualone	–	–	–	–	N/A
TXH079		31	M	C	Stab	Homicide	20	Black	Y	Y	Methaqualone	Acetaminophen, Hydromorphone, Lidocaine, Methamphetamine, Methaqualone, Noscapine, Papaverine, Phenytoin	–	N/A	Methamphetamine	Methamphetamine	–
TXH080		15	M	C	Shot	Homicide	5	Black	Y	Y	–	Acetaminophen, Methamphetamine, Methaqualone	Methaqualone	Methaqualone	Diphenhydramine	Diphenhydramine Methaqualone	N/A
TXH081		23	M	C	Assault	Homicide	20	Black	Y	Y	Methaqualone	Methaqualone, Thiabendazole	Methaqualone	Methaqualone	–	–	–
TXH082		25	M	C	Assault	Homicide	20	Black	N	Y	Methaqualone	Acetaminophen, Codeine, Hydrocodone, Methamphetamine, Methaqualone, Thiabendazole	–	–	–	–	N/A
TXH084		26	M	C	Shot	Homicide	20	Black	Y	Y	Acetaminophen	Acetaminophen, Amphetamine, Diphenhydramine, Lidocaine, Methamphemine, Methaqualone, Thiabendazole	Methaqualone	Diphenhydramine Methaqualone	Methamphetamine Diphenhydramine Methaqualone	Methaqualone	N/A
TXH085		33	M	A	Stab	Homicide	20	Black	N	Y	–	Acetaminophen, Quinine, Verapamil	–	–	–	–	N/A
TXH086		25	M	C	Shot	Homicide	20	Black	Y	Y	–	Acetaminophen, Amphetamine, Methamphetamine, Methaqualone	Methaqualone	Methaqualone	Methamphetamine Diphenhydramine Methaqualone	–	N/A
TXH087		41	M	C	Drown	Accident	20	Black	N	Y	–	Amphetamine, Methamphetamine, Methaqualone, Thiabendazole	–	–	–	–	–
TXH088		28	M	C	Shot	Homicide	20	Black	Y	Y	–	Acetaminophen, Amphetamine, Diphenhydramine, Lidocaine, Methamphetamine, Methaqualone, Midazolam, O-Desmethyltramadol, Tramadol	–	–	Lidocaine	–	N/A
TXH089		33	M	A	Stab	Homicide	20	Black	N	Y	–	Acetaminophen, Methamphetamine, Methaqualone	–	–	–	–	N/A
TXH090		26	M	A	Assault	Homicide	20	Black	Y	Y	Methamphetamine, Methaqualone	Acetaminophen, Amphetamine, Diazepam, Diphenhydramine, Methamphetamine, Methaqualone	–	–	Lidocaine Ketamine	Lidocaine Ketamine	N/A
TXH091		35	M	A	Stab	Homicide	20	Black	Y	Y	–	Methamphetamine, Quinine, Verapamil	Methaqualone	Methaqualone	Diphenhydramine Methaqualone	THC-OH Methaqualone	N/A
TXH092	P1	40	M	A	Assault	Homicide	10	Black	N	Y	–	Methamphetamine, Methaqualone, Quinine	–	–	–	–	–
	P2						10			Y	–	Acetaminophen, Methaqualone					
	D1						10			Y	–	Acetaminophen					
	D2						10			Y	–	Methamphetamine, Methaqualone					
TXH093		30	M	A	Electrocution	Accident	20	Black	N	N	–	–	–	–	–	–	N/A
TXH094		24	M	A	Stab	Homicide	20	Black	N	N	–	–	–	–	–	–	N/A
TXH095		18	M	A	Shot	Homicide	20	Black	Y	Y	–	Amphetamine, Methamphetamine	Methaqualone	Diphenhydramine Methaqualone	Methamphetamine Diphenhydramine Methaqualone	–	N/A
TXH096		32	M	A	Shot	Homicide	20	Black	N	N	–	–	–	–	–	–	N/A
TXH097		27	M	A	Hang	Suicide	20	Black	N	N	–	–	–	–	–	–	N/A

CAS NO	Segments	Age	Sex	Ethnicity	Apparent cause of death	Suspected Manner of Death	Weight (mg)	Hair colour	Substance Present in other matrices	Substance Present in hair	Washes	Hair	Femoral Blood	Heart Blood	Urine	Vitreous Humor	Bile
TXH098		23	M	C	Shot	Homicide	20	Black	Y	Y	–	Acetaminophen, Amphetamine, Diphenhydramine, Methamphetamine, Thiabendazole	Diphenhydramine Methaqualone	Methaqualone	Methamphetamine Diphenhydramine Methaqualone	Diphenhydramine THC-OH Methaqualone	N/A
TXH099		58	M	A	Shot	Homicide	20	Black	N	Y	–	Caffeine, Sildenafil	–	–	–	–	N/A
TXH100		37	M	C	Stab	Homicide	20	Black	Y	Y	–	Acetaminophen, Amphetamine, Caffeine, Methamphetamine, Verapamil	–	Diphenhydramine Methaqualone	Methamphetamine Diphehydramine Methaqualone	–	N/A
TXH101	P1	52	M	C	Shot	Homicide	20	Black	Y	Y	–	Acetaminophen, Caffeine, Diphenhydramine, Methaqualone	Dephenhydramine Methaqualone	Diphenhydrimine Methaqualone	Diphenhydramine Methaqualone	Methaqualone	N/A
	P2						20			Y	–	Acetaminophen, Caffeine, Methaqualone, Sildenafil					
	D1						20			Y	–	Benzocaine, Carbamazepine, Diphenhydramine, Methaqualone					
	D2						20			Y	–	Acetaminophen, Methamphetamine, Methaqualone					
TXH102		25	M	A	Assault and Stab	Homicide	20	Black	Y	Y	–	Sulfadoxine	–	Methamphetamine Methaqualone	Methamphetamine	–	N/A
TXH103		30	M	A	Assault	Homicide	20	Black	Y	Y	–	Acetaminophen, Amphetamine, Diphenhydramine, Hydrocodone, Lidocaine, Maprotiline, Methamphetamine, Methaqualone, Nortriptyline, Protriptyline	N/A	–	Methamphetamine Diphenhydramine	–	Diphenhydramine
TXH104		32	M	A	Stab	Homicide	20	Black	Y	Y	–	Caffeine, Katamine	–	Ketamine	Ketamine	Katamine	N/A

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