Investigating the role of commonly misused drugs in suspected unnatural and/or unexplained deaths in Cape Town in 2022

by

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DECLARATION

I, **Murendwa Success Munarini**, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

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DEDICATION

I dedicate this dissertation to the Lord God Almighty for giving me hope and courage to go after my dreams. He gave me strength in my darkest days.

It is wholeheartedly dedicated to my beloved parents, who have been my source of inspiration and giving me strength when I thought of giving up, who continually provide their moral, spiritual, emotional, and financial support where possible. They gave life to me and groomed me to be the strong and disciplined person I am today. Thank you so much for constantly reminding me of the goals I need to achieve. I am where I am today because of your love and contribution.

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ABSTRACT

Background: Unnatural deaths, which include homicides, suicides, and accidental deaths, often require toxicological analysis to assist with determining the cause of death. In 2022, the Forensic Toxicology Unit within the Western Cape Forensic Pathology Service piloted a targeted drugs analysis at Salt River Mortuary in Cape Town. This permitted an assessment of commonly misused drugs in suspected unnatural and/or unexplained deaths, which was previously not feasible, given the backlogs of National toxicological analyses.

Aim: To assess toxicological findings of a targeted LC-MS/MS drugs of misuse panel in suspected unnatural death cases in the west metropole of Cape Town, Western Cape.

Methods: A retrospective, descriptive study was conducted on all cases for which specimens were submitted to the Forensic Toxicology Unit between 1 January 2022 and 31 December 2022 for a targeted drugs analysis of 31 analytes using LC-MS/MS. Blood alcohol and toxicology results from the National Forensic Chemistry Laboratory were also compared within the cohort.

Results: The Forensic Toxicology Unit analysis was requested in 735 cases, with qualitative (n=723, 98.4%) and quantitative (n=108, 14.7%) analyses performed. Drugs were detected in 382 (52.8%) of the 723 cases analysed. The most frequently detected drugs were acetaminophen (24.8%), methamphetamine (17.6%), amphetamine (14.4%), methaqualone (13.8%) and THC-COOH (12.9%). Blood alcohol analysis was requested from the National Forensic Chemistry Laboratory in 541 cases (73.6%), and ethanol was detected (≥0.01 g/100mL) in 138 (25.6%) of these cases, with a mean positive BAC of 0.14 ± 0.02 g/100 mL (range: 0.01-0.54 g/100 mL).

Conclusion: This study revealed that commonly misused drugs were frequently present in suspected unnatural and/or unexplained deaths admitted to Salt River Mortuary in Cape Town. However, given that only a portion of cases were submitted for analysis, and that the panel was limited in scope, it does not represent the full landscape of drug exposure in unnatural deaths. Nonetheless, this study represents the first comprehensive data on drugs (other than alcohol) in post-mortem toxicology casework in South Africa, using validated methodology.

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6-AM	6-acetylmorphine
BAC	blood alcohol concentration
CBD	cannabidiol
CDA	Central Drug Authority
CO	carbon monoxide
DEA	Drug Enforcement Administration
DOA	drugs of abuse
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
GBD	global burden of disease
GC-MS	gas chromatography mass spectrometry
HREC	Human Research Ethics Committee
LC-MS	liquid chromatography mass spectrometry
LC-MS/MS	liquid chromatography-tandem mass spectrometry
MDA	3,4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
NHLS	National Health Laboratory Services
NPS	new psychoactive substances
SA	South Africa
SACENDU	South African Community Epidemiology Network on Drug Use
SADPI	South African Drug Policy Initiative
SAPS	South African Police Service
SUDA	sudden unexpected death in adults

SUDI	sudden unexpected death in infants
THC	tetrahydrocannabinol
THC-COOH	11-nor-9-carboxy-delta^{9}-tetrahydrocannabinol
UNODC	United Nations Office on Drugs
UPLC-MS/MS	ultra performance liquid chromatography tandem mass spectrometry
WCGHW	Western Cape Government Department of Health and Wellness

1. Chapter 1: Background and literature review

1.1. Background

Drug misuse is frequent (Finkelstein et al., 2017) and continues to be a public health concern worldwide due to its role in morbidity and mortality (Degenhardt & Hall, 2012; Hill & Thomas, 2020). Suspected unnatural and/or unexplained deaths such as homicides, suicides, and accidents may require toxicological analysis to assist with determining the cause of, and circumstances surrounding death (Pan et al., 2019). Owing to an increase in the number of unnatural deaths each year (Western Cape Government, 2018) and the high need for fast, reliable, accurate and comprehensive toxicological analyses in South Africa (SA); application of efficient and sensitive modern screening and confirmatory techniques is an ever-increasing requirement in post-mortem toxicology (Drummer, 2007; Peters et al., 2007).

In the west metropole of the City of Cape Town, SA, post-mortem toxicological investigations were historically conducted by the National Department of Health's Forensic Chemistry Laboratory, now part of the National Health Laboratory Services (NHLS) (NHLS, 2022). However, toxicological investigations other than blood alcohol analysis are not routinely performed in the majority of unnatural deaths by the Forensic Chemistry Laboratory due to resource constraints (James, 2015; Liebenberg et al., 2016, NHLS, 2022). Other areas of concern in the toxicological postmortem context of SA include the delays in issuing a cause of death and court outcomes, lack of feedback to the next of kin and unreliable toxicological results following prolonged storage periods (analytes may degrade following many years of not being analysed) (James, 2015). In addition to the large backlog of toxicological analyses at the National laboratories, the lack of comprehensive toxicological data, especially in the post-mortem setting, is a major drawback. Forensic Toxicology is historically a poorly developed profession in SA, and this has unfortunately hindered the country's ability to monitor and comprehend the entire scope of drug misuse and its relationship to unnatural deaths.

In 2022, the Forensic Toxicology Unit within the Western Cape Forensic Pathology Service, piloted a validated drugs of abuse (DOA) liquid chromatography mass spectrometry (LC-MS/MS) assay for use in post-mortem toxicology investigations. This study assessed the results from the

pilot project of the assay performed in cases autopsied at Salt River Mortuary, to identify patterns and trends in the presence of drugs in suspected unnatural and/or unexplained deaths in the region.

1.2. Literature review

1.2.1. Post-mortem toxicology

Forensic toxicology refers to the detection, identification, quantification and interpretation of drugs, poisons, and any chemical substance, in biological specimens, for medico-legal purposes (Drummer & Gerostamoulos, 2013). These analyses are typically achieved through instrumental analyses using immunoassays, liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) (Wu et al., 1993; Schütz et al., 2006; Tominaga et al., 2015). This discipline is required not only for post-mortem cases but also for the determination of drugs in hospitalised patients admitted following a suspected poisoning; drug-facilitated crimes in which drugs are used to intoxicate or sedate; driving under the influence of drugs; and other aspects of human performance alteration or impairment (Drummer & Gerostamoulos, 2013).

Post-mortem toxicology is one of the sub-disciplines of forensic toxicology that aims to assist in establishing the role of drugs and toxicants in the contribution to, or cause of, death (Skopp, 2010). These analyses are not only crucial in the determination of the cause of death and circumstances surrounding unnatural deaths but also in detecting frequencies of commonly misused drugs in public health (Tominaga et al., 2015). The purpose and comprehensiveness of conducting post-mortem toxicological examinations will however vary depending on the case at hand (Drummer, 2007). That is, it may be necessary to test for a specific drug in certain circumstances, and a comprehensive toxicological analysis may be appropriate in other cases.

The selection of an appropriate specimen in post-mortem toxicology is further of the utmost importance (Skopp, 2004; Drummer, 2007). Forensic pathologists routinely collect femoral whole blood and urine specimens for the investigation of commonly misused drugs in unnatural deaths (Drummer & Gerostamoulos, 2002; Kerrigan, 2013; Liebenberg et al., 2016). Other specimens such as vitreous humour, hair, bone, brain, fats, and muscles may be used in certain special cases and depending on the availability of specimens, scope of testing, and the suspected drug or chemical (Drummer, 2004). It should be highlighted that excretory samples such as urine are

typically best for qualitative screening purposes to investigate prior exposure to a drug (Bailey et al., 2016).

In SA, the collection of biological samples for downstream toxicological analysis is at the discretion of the attending forensic pathologist and this usually depends on the circumstances of the death, the medical and social history that is provided, and whether pathological causes of death are discovered during an autopsy (National Health Act, 2003; Auckloo & Davies, 2019).

Detecting and interpreting analytes in post-mortem specimens bring further challenges in comparison to clinical specimens (Drummer, 2004). Some drugs are prone to alteration depending on the storage conditions (including temperature and available preservative). Post-mortem changes, such as post-mortem synthesis, degradation or redistribution, may further hinder the interpretation of the results (Robertson & Drummer, 1995; Moriya & Hashimoto, 1996; Drummer, 2013). Forensic toxicologists and pathologists should therefore be aware of various factors affecting the analysis of specimens and interpretation of the subsequent analytical results.

1.2.2. Global prevalence of drugs

Drug misuse and abuse permeates every aspect of our societies globally, leading to many deaths each year, and further results in economic and public health implications (Liebenberg et al., 2016). In 2020, globally 1 in every 18 persons between the ages of 15 to 64 years, majority of whom were male, in a projected 284 million people (5.6% of the population), had reportedly taken a drug in the previous 12 months (UNODC, 2022). Additionally, in the European Union, it is estimated that 83.4 million adults (aged 15 to 64) have used illicit drugs, with more men (50.5 million) than women (33.0 million) reporting use (EMCDDA, 2022). Cannabis remains the world's most used drug, followed by stimulants such as amphetamines, ecstasy/methylenedioxymethamphetamine (MDMA), and cocaine (EMCDDA, 2022; UNODC, 2022). In the Southern part of Africa, heroin and cocaine trafficking continues to be a major concern (UNODC, 2022).

The European Union have documented roughly 5,800 overdose fatalities pertaining to the use of illegal drugs in 2020, which amounted to an expected mortality rate of 17.5 per million adult population (EMCDDA, 2022). Data from the Global Burden of Disease (GBD) have estimated 494,000 deaths from drug related causes worldwide and 30.9 million years of 'healthy' life lost due to premature death and morbidity in 2019 (Vos et al., 2020). Other global studies have reported on

opioids/opiates, cannabis, and amphetamine type stimulants as being the most frequently used drug classes resulting in unnatural deaths (Bucello et al., 2010; Calabria et al., 2010; Nelson et al., 2010; Degenhardt et al., 2011). Although the global prevalence of opioids is quite low as compared to other drugs of misuse (such as cannabis and stimulants), they are frequently associated with high mortality rate including overdoses (UNODC, 2022).

The majority of post-mortem toxicological investigations worldwide indicate that about half or more of the reported homicide and suicide victims test positive for recreational drug use (Darke et al., 2006; Darke et al., 2009; Darke, 2010), thereby indicating a high prevalence of drug use globally. The use of recreational drugs has been associated with an increased risk of mortality and morbidity (Rivara et al., 1997).

Road traffic fatalities while under the influence of drugs is another ongoing global pandemic as highlighted in a study by Drummer et al. (2004), which investigated fatal injuries in drivers following road traffic crashes in Australia. Drugs were detected in 23.5% of cases, which included cannabinoids (13.5%), opioids (4.9%), stimulants and benzodiazepines (4.1%). Another study by Pan et al. (2019) investigating drug abuse in post-mortem specimens in Shanghai, China, reported similar findings wherein drugs were detected in 60% of the cases included, although this included 6,787 cases where the three leading causes of death were traffic accidents, sudden death and poisoning. These included amphetamine-type stimulants (83.2%), including methamphetamine (81.0%), ecstasy (2.2%), and methcathinone (0.05%), reportedly the most frequently misused drugs (Pan et al., 2019). Other detected drugs of abuse were heroin (6.2%), ketamine (5.3%), cannabis (4.5%), and cocaine (0.8%) (Pan et al., 2019).

The data pertaining to the prevalence of other drugs of abuse such as hallucinogens, inhalants, and non-medical use of benzodiazepines in the postmortem setting are reportedly scarce compared to other drugs such as opioids, cannabis, amphetamine, cocaine and new psychoactive substances (NPS) (Degenhardt & Hall, 2012). The most comprehensive and up to date information comes from advanced nations in Europe, North America, and Australia (UNODC, 2022). Countries such as Africa, Latin America, Caribbean and Asian regions have little to no toxicological postmortem data (Peacock et al., 2018). These low- or middle-income countries usually have harsh drug laws and are more susceptible to political and societal instabilities (Peacock et al., 2018).

Opioids/opiates, stimulants and NPS were further reported below since their use have been associated with a higher mortality rate worldwide.

1.2.2.1. Opiates and Opioids

The opioid crisis (related to misuse of opiates and opioids, either illicit or prescribed) is a growing concern worldwide, resulting in 77% of all drug-related deaths in 2022 (UNODC, 2022). These group of substances continues to be the most lethal class of drugs globally (EMCDDA, 2022; UNODC, 2022). Opioids were identified in an estimated 74% of fatal overdoses recorded in the European Union, although it should be highlighted that multiple drugs were frequently reported in toxicology reports from suspected drug-induced deaths (EMCDDA, 2022). Heroin remains the most commonly misused opioid globally, while in Africa, there has been evidence of a rise in non-medical tramadol use and concomitant consequences in recent years (Volkow, 2014; UNODC, 2022). The UNODC report of 2022 has documented the rise in opiate users in some parts of Africa, including SA (UNODC, 2022).

Fentanyl is a highly potent opioid which continues to increase overdose fatalities to new peaks in North America, with 91,799 overdose deaths in the United States in 2020 and a preliminary prediction of 107,622 deaths in 2021 (UNODC, 2021). It is also worth noting that opioids other than heroin, such as methadone and, to a lesser extent, buprenorphine and oxycodone, were linked to a significant proportion of overdose deaths in several countries (EMCDDA, 2022). In the first year of the coronavirus disease (COVID-19) epidemic, Canada recorded a 95% rise in opioid overdose mortality (UNODC, 2021).

1.2.2.2. Central nervous system stimulants

The usage of cocaine, ecstasy, and amphetamines by adults globally was estimated at 3.5 million, 2.6 million, and 2 million, respectively, in 2021 (EMCDDA, 2022). Throughout Western and Southern Africa, cocaine use is rather common and seems to be rising in the continent, while methcathinone is widespread in East Africa (Degenhardt et al., 2008; Carrier & Klantschnig, 2012). While North America has the highest prevalence of amphetamine-type stimulant misuse, East and South-East Asia have the biggest number of amphetamine users (UNODC, 2022).

Stimulant drugs were identified as the drug class responsible for the vast majority of drug-related deaths in 11% of countries, including SA (UNODC, 2022). In addition, amphetamine-type

stimulants (methamphetamine, amphetamine and ecstasy) accounted for 7% of all drug-related deaths, followed by cocaine (4%) in 2022. These findings were in keeping with a post-mortem study by Tominaga et al. (2015) in the Southern half of Osaka City, Japan, who investigated drugs of abuse using immunoassay, LC-MS and GC-MS for screening. Their study detected drugs in 16.2% of cases, with amphetamine as the most predominant drug (Tominaga et al., 2015).

1.2.2.3. New psychoactive substances

In 2020, around 370 previously reported NPS were identified on the market, which then increased to 618 in 2021 (UNODC, 2022). Furthermore, between 2010 and 2021 NPS drugs on the market rose from 162 to 618 unique analytes, of which 87 were newly identified in 2021 alone (UNODC, 2022). While synthetic stimulants and cannabinoids dominated the market in 2015, there was a rise in synthetic opioids by 2021 (UNODC, 2022). These are a wide range of synthetic drugs that have similar structures to traditional drugs and are altered/adulterated to evade legislative control. The most widely detected NPS are synthetic cannabinoid receptor agonists and synthetic cathinones (UNODC, 2022). Since 2008, 224 novel synthetic cannabinoids were discovered in Europe (EMCDDA, 2022). Germany, Hungary and Turkey were amongst other countries with high synthetic cannabinoid mortalities (EMCDDA, 2022). Although there are little statistical data on both cannabinoids and cathinones, these NPS are mostly reported in Southern part of Africa (UNODC, 2022). In the Eastern Europe and Central Asian regions, the rise in the use of synthetic NPS has been particularly noticeable throughout the period 2015–2020 (UNODC, 2022).

It is evident from the literature that there exists a wide range of fatal substances globally, including cannabis, amphetamine-type stimulants, opioids, cocaine, benzodiazepines and at some extent new psychoactive drugs. It should be emphasised that the prevalence of direct drug-related deaths among the general population varies significantly by geography.

1.2.3. Drug misuse in South Africa

SA is known to be the largest trader of illicit drugs in sub-Saharan Africa (Nel, 2013; Thomson, 2013; Lehohla, 2014). The high availability of drugs as a consequence of volatile social, economic and political landscapes in SA has made the country more susceptible to drug-related deaths (Peltzer et., 2010). Data on drug misuse and abuse, and more so in post-mortem toxicology, is scarce in SA. Previous studies include nationwide survey-style studies, post-mortem reports, school

surveys, police arrests, and drug seizures (Donaldson et al., 2006). Statutory bodies such as the Central Drug Authority (CDA), South African Drug Policy Initiative (SADPI) and South African Medical Research Council (SAMRC) including the South African Community Epidemiology Network on Drug Use (SACENDU) do not report on post-mortem toxicological data.

SA drug of abuse statistics for 2020 showed that about 8.9% of the population aged between 15 to 64 years used cannabis, opiates (0.4%), cocaine (0.8%), and amphetamine-type stimulants (0.9%) (CDA, 2021/22). The primary substances of abuse at admission to most government-funded treatment centres in 2020 were cannabis (19.9%), methamphetamine (5.2%), crack/cocaine (7.8%), methaqualone (2.6%), heroin/opiates (5.5%), and prescription medications (2.8%) (SACENDU, 2022). The most primary substance of use reported in the Western Cape treatment centres in 2021 were methamphetamine (53%), cannabis (24%) and heroin (11%) (SACENDU, 2022). In the Gauteng region, heroin (29%), cannabis (27%), methamphetamine (17%) and methcathinone (8%) were mostly reported (SACENDU, 2022). Despite regional variations in drug usage in SA, cannabis remains the primary substance of choice among individuals younger than 20 years across the country (Peltzer et al., 2010; , SACENDU, 2022).

1.2.3.1. Post-mortem toxicology in South Africa

In 2021/22, the South African Police Service (SAPS) reported 293,217 assaults, 25,204 homicides, 18,548 attempted homicides, and 46,548 sexual offences nationwide (SAPS, 2021/22). Cannabis, mandrax (methaqualone and diphenhydramine), 'tik' (methamphetamine), cocaine and heroin have been documented to be the most frequently abused drugs associated with high mortality according to SAPS annual report of 2021/22 (SAPS, 2021/22).

Driving under the influence of drugs is also a common practice among South Africans (Liebenberg et al., 2019), and is reported to be the leading cause of preventable death in the country (WHO, 2015). Liebenberg et al. (2019) conducted a study reporting on frequently detected drugs impairing drivers in SA, which included amphetamine, cocaine, illicit opiates, benzodiazepines, sedatives and medicinal opioids. It should be noted that these statistics might not be a true representation of the overall road traffic fatalities (as a result of drug use) in SA, as the country battles from a lack of resources and expertise to monitor and evaluate the situation (Degenhardt et al., 2011; UNODC, 2021).

Another similar toxicological study in violent fatalities conducted at Salt River Mortuary in the west metropole of Cape Town detected drugs in 63 of 104 included cases (Auckloo & Davies, 2019). The most commonly detected substances were a combination of methamphetamine, diphenhydramine, and methaqualone (Auckloo & Davies, 2019). Methamphetamine is thought to be a widely used drug in Cape Town and is usually associated with gang-related violence (Peltzer et al., 2010; Auckloo & Davies, 2019). In the Western Cape region of SA, other amphetamine stimulants (such as amphetamine and ecstasy) have also been linked with a large number of fatalities (Peltzer & Phaswana-Mafuya, 2018).

Liebenberg et al. (2016) found that the most frequently used illicit drugs in post-mortem cases admitted at the Pretoria medico-legal laboratory were a combination of heroin (35.2%), cocaine (19.9%), amphetamine-type stimulants (6.4%), cannabis (4.6%), and methaqualone (4.3%). Other substances such as methcathinone are also linked with fatalities and are common in Gauteng (Liebenberg et al., 2016). Drugs such as psilocybin, lysergic acid diethylamide (LSD), phencyclidine, gamma-hydroxybutyrate (GHB) and ketamine were rarely detected (Liebenberg et al., 2016). However, it is recognised that these are drugs that may not be used in the community, may not be targeted in analysis, or rapidly degrade in biological samples before testing.

1.2.4. The South African medicolegal system

Medicolegal death investigations in SA are governed by two important statutes namely, the Inquests Act (Act 58 of 1959) and the National Health Act (Act 61 of 2003). The National Health Act defines unnatural death as any death due to chemical or physical influences (including drug and chemical toxicity, violence, road traffic accidents etc.), an act of commission or omission, sudden and unexpected or unexplained circumstances, and procedure-related deaths as defined by the Health Professions Act (no. 56 of 1974).

The Inquest Act mandates that all deaths suspected to be due to other than natural causes shall be investigated (Inquest Act, 1959). As a result, forensic pathologists are responsible for conducting an autopsy to determine the cause of death (National Health Act, 2003). The final manner of death is, however, determined by the magistrate in Inquest or Criminal Court proceedings after reviewing all supporting evidence (Inquests Act, 1959). The pathologist determines whether to collect specimens for toxicological analyses, usually based on the circumstances surrounding death, the

medical and social history that is known, and whether pathological causes of death are discovered during autopsy (Liebenberg et al., 2016).

1.2.5. Rationale

There is limited data concerning drugs in death cases in SA. One of the key reasons for this are the historic backlogs in toxicological analyses within the National Forensic Chemistry Laboratories (Liebenberg et al., 2016). In an attempt to improve the forensic toxicology profession in South Africa, the Forensic Pathology Service within the Western Cape Government Department of Health and Wellness (WCGHW), invested in the development of the Forensic Toxicology Unit to provide scientific support in medico-legal death investigations within the Western Cape Province.

In 2022, the Forensic Toxicology Unit rolled out a targeted qualitative and quantitative ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis for 31 commonly misused drugs in post-mortem blood and urine to the Salt River Mortuary within the province, to pilot the assay before provincial release. This project aims to review the data obtained from the casework analyses to determine the frequency of detected drugs and the types of cases for which drug analyses were requested.

1.2.6. Aims and objectives

The aim of this study was to profile the toxicological findings of a targeted UPLC-MS/MS panel for 31 common drugs in suspected unnatural and/or unexplained death cases in the west metropole of Cape Town in 2022.

This was achieved by completing the following objectives:

i. A retrospective review of the Forensic Toxicology Unit's Forensic Sample Repository (HREC: R006/21) and identifying and documenting all suspected unnatural and/or unexplained death cases in which specimens were submitted for this analysis in 2022.
ii. Conducting and reporting on descriptive and inferential statistical analyses of collected variables to better understand the characteristics of these cases and the frequencies of drugs detected.

2. Chapter 2: Research methods

2.1. Study design

A retrospective descriptive study was conducted on all suspected unnatural and/or unexplained deaths for which biological specimens from unnatural death cases were submitted to the Forensic Toxicology Unit for the targeted drugs analysis between 1 January and 31 December 2022. There were no exclusion criteria applied.

2.2. Study setting

Salt River Mortuary covers the west metropole of Cape Town (with a population of over 1,937,380) (Statistics South Africa, 2012), which includes Western, Southern, Klipfontein, and Mitchells Plain districts and is one of 16 Forensic Pathology Service mortuaries in the province. It is one of the busiest mortuaries in the Western Cape (Reid et al., 2020), with approximately 4,000 suspected unnatural death admissions annually (Western Cape Forensic Pathology Service, 2022).

2.3. Data collection

The Forensic Toxicology Unit's internal database, the Forensic Sample Repository, was used to identify all relevant cases submitted for drugs analyses. Post-mortem reports of included cases were reviewed to collect the remaining variables and confirm the database information. Collected variables included demographic profile (age and sex), hospitalisation, alleged circumstances of death, cause(s) of death, specimen(s) submitted for analysis, and toxicological results from both the Forensic Chemistry Laboratory (where they were submitted) and Forensic Toxicology Unit.

Age was categorised into nine (9) groups, namely: infant (<1 year), toddler (1-4 years), child (5-9 years), juvenile (10-14 years), adolescent (15-19 years), young adult (20-29 years), middle-aged adult (30-45 years), older adult (46-74 years) and elderly (≥75 years), similar to previous studies (Gradisar et al., 2013, Lin et al., 2019). Causes of death were also grouped into categories: 'Asphyxia' included asphyxia and choking-related deaths; blunt force and sharp force trauma deaths were classified as 'Trauma'; and all deaths due to carbon monoxide (CO) and smoke inhalation and/or burns were grouped into 'Burns and/or CO toxicity'. Less frequent causes were classified as 'Other', and included hypothermia, procedure-related deaths, electrocution and combination deaths. 'Combination' deaths were instances where more than one cause of death was

noted (e.g., blunt force trauma and drowning; hanging and pesticide ingestion; blunt force trauma and asphyxia; sharp force trauma and burns; assault and myocardial infarction).

2.4. Toxicology analyses

Specimens were submitted to the Forensic Toxicology Unit by forensic pathologists following autopsy and prepared and analysed on a Waters Xevo TQD UPLC-MS/MS system for 31 analytes. The analytes, internal standards and limits of detection and quantitation are included in Appendix A and B. The method was validated in accordance with local (SANAS TG41) and international (ASB Standard 036) guidelines for qualitative and quantitative analysis in urine and blood, respectively.

Given that the Forensic Toxicology Unit targeted panel was limited in scope, pathologists would typically submit additional specimens for blood alcohol analysis and occasionally other drug analyses to the Forensic Chemistry Laboratory. Submissions and results (where available) were also assessed in this study.

2.5. Data management

The study used retrospective data from the Forensic Sample Repository of the Forensic Toxicology Unit and postmortem reports. Access to this data was obtained from the Forensic Toxicology Unit and the Division of Forensic Medicine and Toxicology, Department of Pathology, University of Cape Town. The data contained was of a sensitive nature and confidentiality of this data was maintained. Data within the Forensic Sample Repository does not contain identifiable information (apart from the death case number). Once cases were identified and key variables were documented, the research database was anonymised, and no identifying information was retained related to the decedents. Data were stored in an access-controlled database on SharePoint, that only the student and supervisors had access to.

2.6. Data analyses

Data were captured using Microsoft® Office Excel® and analysed with the Statistical Package for Social Sciences (SPSS; Ver 28). Frequencies were used to represent variables including demographic profile (gender and age categories), cause(s) of death, manner of death, hospitalised (yes/no) and drugs of misuse. Descriptive statistics (mean, median, range and standard deviation)

were conducted for age, blood alcohol concentrations and quantitative drug analyses. Pearson Chi-Square was used to compare statistically significant differences between variables.

2.7. Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki (2013), and ethical approval was obtained from the Human Research Ethics Committee (HREC) (HREC: 266/2023) (Appendix C). The Forensic Sample Repository has HREC ethical approval (HREC: 006/2021), and approval to use this data was obtained from the Forensic Toxicology Unit Chief Toxicologist, as well as the Head of the Division of Forensic Medicine and Toxicology, University of Cape Town.

3. Chapter 3: Results

3.1. Case characteristics

In 2022, 4,204 suspected unnatural and/or unexplained death cases were admitted to Salt River Mortuary, of which biological samples from 715 (17.0%) cases were submitted for a targeted drug analysis at the Forensic Toxicology Unit. An additional 20 cases were submitted from Tygerberg Mortuary, resulting in a total of 735 cases included in this study.

The majority of the decedents in the cohort were male (69.5%) (Table 1). The mean age was 31 years (range: 0–86 years; SD: ±18.8; median: 32 years), with most decedents in the middle-aged category (30-45 years) (n=285, 38.8%). Only a few decedents were hospitalised prior to death (n=122, 16.6%), which were predominantly drug toxicity (48% of toxicity deaths) and trauma (23% of traumatic deaths) cases (Table 1).

The cause of death was under investigation in almost a third of the cases (n=217, 29.5%) (Table 1). Overall, the most frequently recorded 'known' cause of death was hanging (n=137, 18.6%), followed by trauma (n=79, 10.7%) and drug toxicity-related deaths (n=64, 8.7%). Less frequent cases were categorised as 'other' (Table 1) and consisted of combination deaths (n=5, 0.7%), and one case each (0.1%) of electrocution, hypothermia and procedure-related death.

Asphyxia, drug toxicity, and sudden unexpected deaths in infants (SUDI) were approximately equally distributed between males and females. However, males were the predominant victims in drowning (n=19, 90.5%), hanging (n=115, 83.9%), burns and CO toxicity (n=25, 80.6%), and gunshot wound (n=23, 79.3%) cases (Table 1). In the infant age category (<1 year) (n=108), most cases were recorded as 'SUDI' (n= 51, 47%) by the pathologist, with 43 (39.8%) cases still under investigation at the time of the study. Across the age categories, the cause of death was noted as 'under investigation' in 18-50% of the cases. Statistically significant differences (p<0.001) were noted between the cause of death and sex, age, hospitalised (yes/no), and manner of death.

The alleged manner of death was under investigation in almost half of the cases (n=344, 46.8%) (Table 1). Suicide was suspected in almost a third of the cases (n=211; 28.7%), followed by accidents (n=92; 12.5%) and homicides (n=66; 9%). Among the alleged suicides, hanging (n=134, 63.5%) was the most frequently recorded cause of death.

Table 1: Characteristics of cases submitted for targeted drugs analysis (N=735) at the Forensic Toxicology Unit (FTU) in 2022, according to cause of death

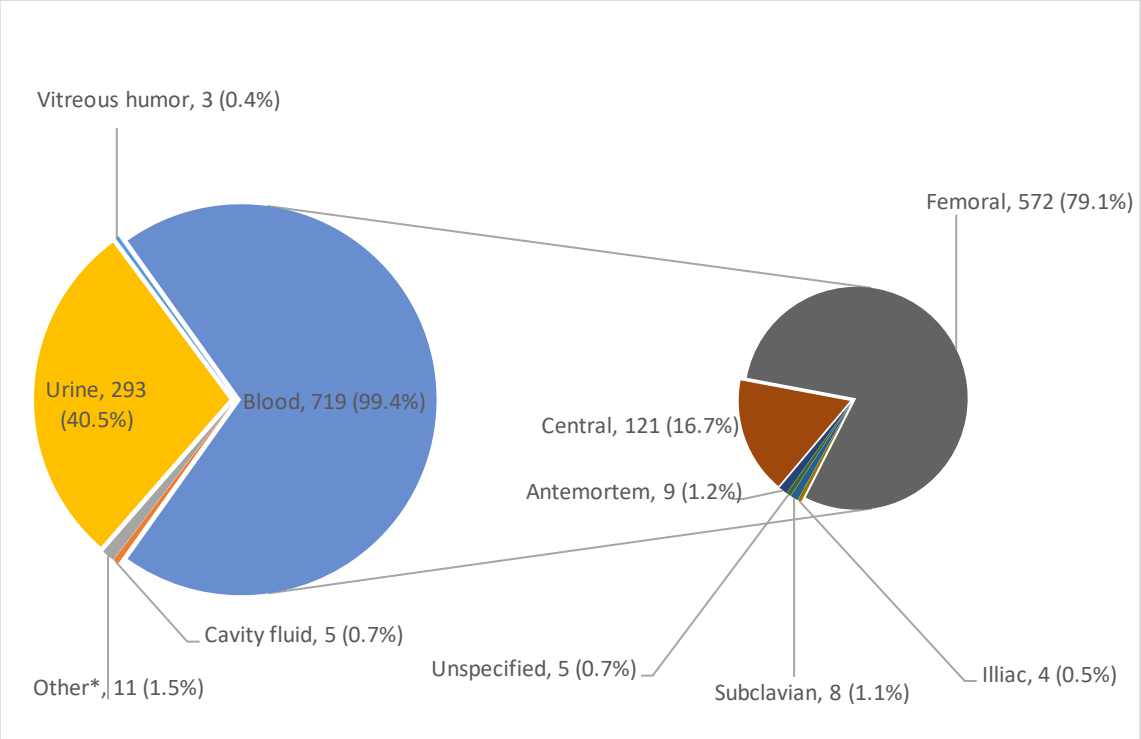
Demographics	Cause of death, n (%)											N=735	*p*-value
	Asphyxia, 23 (3.1)	Burns &/CO toxicity, 31 (4.2)	Drowning, 21 (2.9)	Gunshot wound, 29 (3.9)	Hanging, 137 (18.6)	Natural, 33 (4.5)	Other[a], 8 (1.1)	SUDA, 38 (5.2)	SUDI, 55 (7.4)	Toxicity, 64 (8.7)	Trauma, 79 (10.7)	Under investigation, 217 (29.5)		
Sex														**<0.001**
Female	10 (1.3)	6 (0.8)	2 (0.3)	6 (0.8)	21 (2.9)	11 (1.5)	2 (0.3)	11 (1.5)	23 (3.1)	27 (3.7)	20 (2.7)	79 (10.7)	218 (29.7)	
Male	13 (1.8)	25 (3.4)	19 (2.6)	23 (3.1)	115 (15.6)	22 (3.0)	5 (0.7)	27 (3.7)	31 (4.2)	37 (5.0)	59 (8.0)	135 (18.4)	511 (69.5)	
Unknown	-	-	-	-	1 (0.1)	-	1 (0.1)	-	1 (0.1)	-	-	3 (0.4)	6 (0.8)	
Age (Years)														**<0.001**
< 1	-	-	-	-	-	10 (1.3)	-	-	51 (6.9)	3 (0.4)	1 (0.1)	43 (5.8)	108 (14.7)	
1 – 4	-	1 (0.1)	2 (0.3)	-	-	-	-	-	4 (0.5)[b]	-	-	5 (0.7)	12 (1.6)	
5 – 9	1 (0.1)	-	-	-	-	-	-	-	-	1 (0.1)	-	2 (0.3)	4 (0.5)	
10 – 14	1 (0.1)	-	3 (0.4)	-	3 (0.4)	-	-	-	-	4 (0.5)	1 (0.1)	4 (0.5)	16 (2.2)	
15 – 19	-	1 (0.1)	-	2 (0.3)	12 (1.6)	-	1 (0.1)	2 (0.3)	-	4 (0.5)	3 (0.4)	10 (1.3)	35 (4.7)	
20 – 29	6 (0.8)	5 (0.7)	3 (0.4)	4 (0.5)	34 (4.6)	7 (1.0)	-	7 (1.0)	-	13 (1.8)	18 (2.4)	31 (4.2)	129 (17.5)	
30 – 45	8 (1.1)	17 (2.3)	6 (0.8)	16 (2.2)	63 (8.6)	12 (1.6)	4 (0.5)	20 (2.7)	-	26 (3.5)	36 (4.9)	78 (10.6)	285 (38.8)	
46 – 74	6 (0.8)	7 (1.0)	7 (1.0)	6 (0.8)	25 (3.4)	4 (0.5)	2 (0.3)	8 (1.1)	-	12 (1.6)	15 (2.0)	42 (5.7)	134 (18.2)	
> 75	1 (0.1)	-	-	1 (0.1)	-	-	1 (0.1)	1 (0.1)	-	1 (0.1)	4 (0.5)	2 (0.3)	11 (1.5)	
Unknown	-	-	-	-	-	-	-	-	-	-	1 (0.1)	-	1 (0.1)	
Hospitalised														**<0.001**
Yes	1 (0.1)	1 (0.1)	1 (0.1)	2 (0.3)	8 (1.1)	5 (0.7)	3 (0.4)	5 (0.7)	4 (0.5)	31 (4.2)	18 (2.4)	43 (5.9)	122 (16.6)	
No	22 (3.0)	30 (4.1)	18 (2.4)	27 (3.6)	128 (17.4)	28 (3.8)	5 (0.7)	33 (4.5)	51 (6.9)	33 (4.5)	59 (8.0)	173 (23.5)	607 (82.6)	
Unknown	-	-	2 (0.3)	-	1 (0.1)	-	-	-	-	-	2 (0.3)	1 (0.1)	6 (0.8)	
Alleged manner of death														**<0.001**
Accident	5 (0.7)	20 (2.7)	14 (1.9)	-	-	2 (0.3)	3 (0.4)	-	-	8 (1.1)	37 (5.0)	3 (0.4)	92 (12.5)	
Homicide	9 (1.2)	1 (0.1)	-	18 (2.4)	-	1 (0.1)[c]	2 (0.3)	-	-	-	27 (3.7)	8 (1.1)	66 (9.0)	
Natural	-	-	-	-	-	13 (1.8)	-	1 (0.1)	1 (0.1)	-	-	6 (0.8)	21 (2.9)	
Procedure-related	-	-	-	-	-	-	1 (0.1)	-	-	-	-	-	1 (0.1)	
Suicide	7 (0.9)	3 (0.4)	2 (0.3)	9 (1.2)	134 (18.2)	-	1 (0.1)	-	-	35 (4.8)	6 (0.8)	14 (1.9)	211 (28.7)	
Under investigation	2 (0.3)	7 (1)	5 (0.7)	2 (0.3)	3 (0.4)	17 (2.3)	1 (0.1)	37 (5.0)	54 (7.3)	21 (2.8)	9 (1.2)	186 (25.3)	344 (46.8)	

Acronyms: CO = carbon monoxide; SUDA = sudden unexpected death in adults; SUDI = sudden unexpected death in infants.
[a]Other cause of death: combination (5 cases); hypothermia, procedure-related, electrocution (1 each).
[b]All SUDI cases in the 1-4 year age category were recorded as 1 year-olds.
[c]'Homicide by heart attack' during robbery.

3.2. Postmortem toxicological findings

3.2.1. Biological specimens

One or more specimens were submitted in most cases that were subsequently analysed (n=723, 98.4%), totaling 1,031 submitted specimens overall. Blood was submitted in almost all cases tested (n=719, 99.4%), followed by urine (n=293, 40.5%) (Figure 1). Amongst the blood specimens, femoral (n=572, 79.1%) and central (n=121, 16.7%) blood were commonly submitted (Figure 1). 'Other' specimen types (n=11; 1.5%) included serum (n=8), bladder wash (n=2) and a nasal swab (n=1).



*Serum (n=8), bladder wash (n=2), nasal swab (n=1)

Figure 1: Frequency of specimen types submitted in 723 cases that were analysed at the Forensic Toxicology Unit (FTU) on a targeted LC-MS/MS panel of 31 commonly misused drugs.

3.2.2. Toxicological requests

3.2.2.1. Forensic Toxicology Unit

Of the 735 drug analysis requests submitted to the Forensic Toxicology Unit in 2022, 12 cases (1.6%) were not analysed (Table 2), as they were of poor integrity or unsuitable for the analysis (e.g., bile). The remaining 723 cases (98.3%) were analysed qualitatively, with five cases (0.7%) producing inconclusive results due to sample quality. Of the cases analysed, drugs were detected

in 382 cases (52.8%). Quantitative analyses were performed in 108 (14.9%) of the cases analysed (n=723).

3.2.2.2. Forensic Chemistry Laboratory

Blood alcohol concentration (BAC) analysis by the Forensic Chemistry Laboratory was also requested by pathologists in the majority of the included cases (n=541, 73.6%), while toxicological analyses by the same laboratory were requested in less than one-third of the included cases (n=206, 28.0%) (Table 2). For many cases, it could not be confirmed in the available post-mortem records whether BAC or toxicology from the Forensic Chemistry Laboratory was requested (20.5 % and 44.9% of cases, respectively) (Table 2). Some of the results from both these analyses were outstanding at the time of the study (BAC: n=145, 26.8%; Tox: n=184, 89.3%).

Of the cases submitted for BAC, ethanol was detected (≥0.01 g/100 mL) in 138 cases (25.6%). Toxicology results for other drugs were available for only 22 cases (10.7%), with one or more drugs detected in 15 (68.2%) of these cases (Table 2). Twenty different analytes and/or metabolites, most frequently acetaminophen and methamphetamine (both n=3), were detected in various specimens (blood, urine, bile, eye fluid and stomach contents).

Table 2: Frequency of requests for, and availability of, results of blood alcohol concentration (BAC) and toxicological testing from the Forensic Toxicology Unit (FTU) and Forensic Chemistry Laboratory (FCL), in 2022 (N=735).

Test type	**Requests for analysis**, n (%)			**Results for 'yes' requests**, n (%)				
	Yes	**No**	**Unspecified**	**Detected***	**Not detected***	**Inconclusive***	**Not analysed****	**Outstanding**
FTU Tox Qualitative	735 (100)	-	-	382 (52.8)	336 (46.5)	5 (0.7)	12 (1.6)	-
FTU Tox Quantitative	108 (14.7)	-	-	108 (100)	-	-	-	-
FCL BAC	541 (73.6)	43 (5.9)	151 (20.5)	138 (25.6)	255 (47.4)	-	3 (0.6)	145 (26.8)
FCL Tox	206 (28.0)	199 (27.1)	330 (44.9)	15 (7.3)***	7 (3.4)	-	-	184 (89.3)

*(%) are based on the total number of cases analysed (n=723) (i.e., cases that not analysed were excluded).
**Specimens not suitable for analysis (e.g., bile/gastric contents), specimen clotted, insufficient specimen.
***__Analytes detected - 3 cases:__ acetaminophen, methamphetamine__; 2 cases:__ amitriptyline, amitriptyline-N-oxide, chlorpheniramine, diphenhydramine, lamotrigine, lidocaine, methaqualone, nortriptyline, tramadol__; 1 case each:__ amlodipine, amphetamine, benzocaine, ketamine, metoclopramide, norfentanyl, phenytoin, orphenadrine, theophylline

3.2.2.2.1. Forensic Chemistry Laboratory blood alcohol analyses

The mean positive BAC was 0.14 ± 0.02 g/100 mL (range: 0.01-0.54 g/100 mL). Majority of the decedents who tested positive for alcohol were males (n=103, 74.6%) and within the 30-45 years age group (n=63, 45.7%) (Table 3), with a mean BAC of 0.15 g/100 mL and 0.13 g/100 mL, respectively (Figure 2).

Table 3: Descriptive and frequency statistics of sex and age groups against positive blood alcohol concentrations (BACs), and against number of positive drug detections by the Forensic Toxicology Unit (FTU) where BAC was positive, versus where BAC was not detected, not available or not requested.

Demographics	Cases with positive BAC* (N= 138)		Cases positive for drugs detected by Forensic Toxicology Unit (n=382) where: n (%)	
	Total Positive, n (%)	Mean (Range) (g/100 ml)	BAC also positive (n=138)	BAC also not positive / not requested / not available
Sex				
Female	34 (24.6)	0.13 (0.01 - 0.29)	12 (8.7)	95 (15.9)
Male	103 (74.6)	0.15 (0.01-0.54)	57 (41.3)	217 (45.4)
Unknown	1 (0.7)	0.16 (-)	-	1 (0.2)
Age group (Years)				
<1	-	-	-	35 (5.9)
1-4	-	-	-	6 (1.0)
5-9	-	-	-	1 (0.2)
10-14	-	-	-	7 (1.2)
15-19	8 (5.8)	0.08 (0.01-0.17)	4 (2.9)	14 (2.3)
20-29	32 (23.2)	0.14 (0.01-0.31)	16 (11.6)	54 (9.0)
30-45	63 (45.7)	0.14 (0.01-0.50)	33 (23.9)	137 (22.9)
45-74	35 (25.4)	0.17 (0.01-0.54)	16 (11.6)	55 (9.2)
> 75	-	-		3 (0.5)
Unknown	-	-		1 (0.2)

* Positive is ≥ 0.01 g/100 mL

While case numbers for positive BACs according to the cause of death were low (Figure 2), the mean positive concentrations were high for hypothermia (0.25 g/100 mL), natural (0.21 g/100 mL), burns (0.19 g/100 mL), gunshot wound deaths (0.17 g/100 mL) and trauma (0.16 g/100 mL). Positive BACs greater than or equal to 0.25 g/100 mL were mostly observed in victims of burns (n=4), trauma (n=4) and sudden unexpected death in adults (SUDA) (n=2). BACs of greater than 0.40 g/100 ml were only detected in cases that were under investigation at the time of the study, where the final cause of death was not yet concluded.

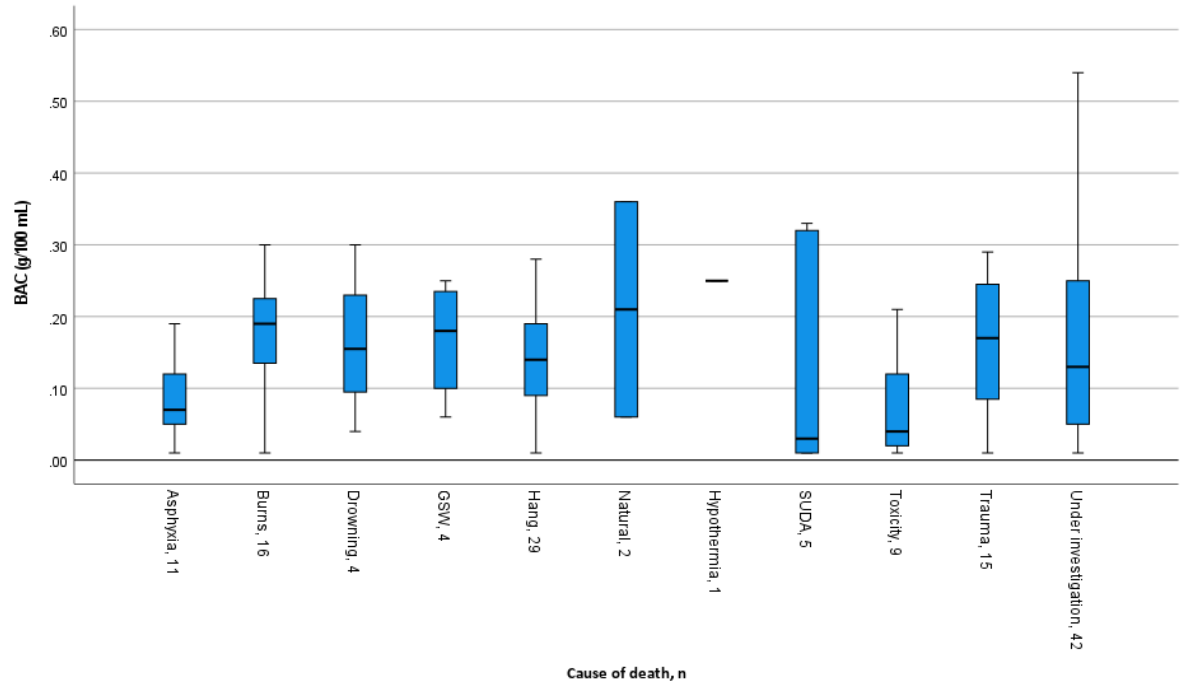


Figure 2: Distribution of positive blood alcohol concentration (BAC) levels (n=138) according to cause of death. Note: The horizontal lines in the blue plot boxes indicate: minimum (1st line), first quartile, median (dark line), third quartile and maximum (last line) values, respectively.

3.2.3. Forensic Toxicology Unit drugs of abuse analyses

3.2.3.1. Qualitative analysis results

Of the 382 positive cases, the most frequently detected drugs were acetaminophen (n=179, 24.8%), methamphetamine (n=127, 17.6%), amphetamine (n=104, 14.4%), methaqualone (n=100, 13.8%) and 11-nor-9-carboxy-delta-tetrahydrocannabinol (THC-COOH) (n=93, 12.9%). Across the 382 positive cases, analytes were detected 1,093 times (i.e., multiple detections for single cases). Drugs such as amitriptyline, clobazam, cocaine, methadone, and oxymorphone were predominantly detected in females (however, case numbers were small) (Table 4). Several illicit drug detections were identified in children and infants including methamphetamine and methaqualone (Table 4). In 138 cases, BAC was also identified as positive, especially with cases of acetaminophen (n=29, 21%), methamphetamine (n=17, 12.3%) and THC-COOH (n=21, 15.2%) detection (Table 4). These were, however, some of the most frequent drugs detected in general.

Table 4: Analytes detected (N=1093) in 723 cases analysed for drugs of abuse (DOA), according to sex, age groups and positive blood alcohol concentrations (BAC).*

Drugs detected (n=382), n (% of 723)	Sex, n (%)			Age group (Years), n (%)										Positive BAC, n (% of 138)
	Female, 316 (29)	Male, 775 (71)	Unknown, 2 (0.2)	<1, 50 (4.6)	1-4, 15 (1.4)	5-9, 6 (0.5)	10-14, 15 (4.6)	15-19, 45 (4.1)	20-29, 234 (21.4)	30-45, 529 (48.4)	46-74, 184 (16.8)	>75, 12 (1.1)	Unknown, 3 (0.3)	
6-Acetylmorphine, 24 (3.3)	4 (0.6)	20 (2.8)	-	-	-	-	-	-	8 (1.1)	13 (1.8)	3 (0.4)	-	-	6 (4.4)
Acetaminophen, 179 (24.8)	54 (7.5)	124 (17.2)	1 (0.1)	24 (3.3)	4 (0.6)	1 (0.1)	5 (0.7)	6 (0.8)	23 (3.2)	70 (9.7)	43 (5.9)	3 (0.4)	-	29 (21)
Alprazolam 29 (4.0)	9 (1.2)	20 (2.8)	-	-	1 (0.1)	-	-	2 (0.3)	6 (0.8)	11 (1.5)	7 (1.0)	2 (0.3)	-	11 (8)
Amitriptyline, 26 (3.6)	14 (1.9)	12 (1.7)	-	1 (0.1)	-	-	-	-	4 (0.6)	8 (1.1)	13 (1.8)	-	-	6 (4.4)
Amphetamine, 104 (14.4)	29 (4.0)	75 (10.4)	-	5 (0.7)	-	1 (0.1)	1 (0.1)	4 (0.6)	25 (3.5)	62 (8.6)	5 (0.7)	-	1 (0.1)	10 (7.3)
Benzoylecgonine, 26 (3.6)	8 (1.1)	18 (2.5)	-	-	-	-	-	-	8 (1.1)	16 (2.2)	2 (0.3)	-	-	12 (8.7)
Clobazam 9 (1.2)	6 (0.8)	3 (0.4)	-	-	-	-	-	-	1 (0.1)	4 (0.6)	3 (0.4)	1 (0.1)	-	-
Cocaethylene, 14 (1.9)	4 (0.6)	10 (1.4)	-	-	-	-	-	-	3 (0.4)	10 (1.4)	1 (0.1)	-	-	11 (8)
Cocaine, 22 (3.0)	5 (0.7)	17 (2.4)	-	-	-	-	-	-	5 (0.7)	15 (2.1)	2 (0.3)	-	-	11 (8)
Codeine, 48 (6.6)	11 (1.5)	36 (5.0)	1 (0.1)	-	2 (0.3)	-	1 (0.1)	1 (0.1)	11 (1.5)	22 (3.0)	11 (1.5)	-	-	10 (7.3)
Diazepam, 28 (3.9)	11 (1.5)	17 (2.4)	-	1 (0.1)	-	-	-	1 (0.1)	7 (1.0)	10 (1.4)	8 (1.1)	1 (0.1)	-	5 (3.6)
Diphenhydramine, 58 (8.0)	18 (2.5)	40 (5.5)	-		1 (0.1)	-	1 (0.1)	2 (0.3)	14 (1.9)	32 (4.4)	7 (1.0)	1 (0.1)	-	8 (5.8)
Fentanyl, 6 (0.8)	3 (0.4)	3 (0.4)	-	-	-	-	-	-	1 (0.1)	4 (0.6)	1 (0.1)	-	-	1 (0.7)
Hydrocodone, 2 (0.3)	1 (0.1)	1 (0.1)	-	-	-	-	-	-	-	1 (0.1)	1 (0.1)	-	-	-
Hydromorphone, 2 (0.3)	1 (0.1)	1 (0.1)	-	-	-	-	-	-	1 (0.1)	1 (0.1)	-	-	-	-
Ketamine, 17 (2.3)	4 (0.6)	13 (1.8)	-	-	-	-	-	-	5 (0.7)	7 (1.0)	4 (0.6)	1 (0.1)	-	1 (0.7)
MDA, 1 (0.1)	1 (0.1)	-	-	-	-	-	-	-	-	1 (0.1)	-	-	-	-
MDMA, 1 (0.1)	1 (0.1)	-	-	-	-	-	-	-	-	1 (0.1)	-	-	-	-
Methadone, 2 (0.3)	2 (0.3)	-	-	-	-	-	-	-	1 (0.1)	1 (0.1)	-	-	-	-
Methamphetamine, 127 (17.6)	33 (4.6)	94 (13.0)	-	9 (1.3)	-	1 (0.1)	1 (0.1)	4 (0.6)	30 (4.2)	71 (9.8)	10 (1.4)	-	1 (0.1)	17 (12.3)
Methaqualone, 100 (13.8)	27 (3.7)	73 (10.1)	-	9 (1.3)	3 (0.4)	1 (0.1)	1 (0.1)	4 (0.6)	19 (2.6)	53 (7.3)	9 (1.3)	-	1 (0.1)	8 (5.8)
Methcathinone, 4 (0.5)	-	4 (0.6)	-	-	1 (0.1)	-	-	-	1 (0.1)	2 (0.3)	-	-	-	2 (1.5)
Morphine, 49 (6.8)	13 (1.8)	36 (5.0)	-	-	2 (0.3)	-	1 (0.1)	-	13 (1.8)	25 (3.5)	8 (1.1)	-	-	9 (6.5)
O-desmethyltramadol, 33 (4.6)	12 (1.7)	21 (2.9)	-	-	-	-	1 (0.1)	-	6 (0.8)	14 (1.9)	11 (1.5)	1 (0.1)	-	6 (4.4)
Oxycodone, 3 (0.4)	2 (0.3)	1 (0.1)	-	-	-	-	-	-	-	1 (0.1)	2 (0.3)	-	-	-
Oxymorphone, 2 (0.3)	2 (0.3)	-	-	-	-	-	-	-	-	1 (0.1)	1 (0.1)	-	-	-
THC, 47 (6.5)	11 (1.5)	36 (5.0)	-	1 (0.1)	-	1 (0.1)	1 (0.1)	7 (1.0)	15 (2.1)	14 (1.9)	8 (1.1)	-	-	10 (7.3)
THC-COOH, 93 (12.9)	15 (2.1)	78 (10.8)	-	-	-	1 (0.1)	-	14 (1.9)	21 (2.9)	44 (6.1)	12 (1.7)	1 (0.1)	-	21 (15.2)
Tramadol, 37 (5.1)	15 (2.1)	22 (3.0)	-	-	1 (0.1)	-	2 (0.3)	-	6 (0.8)	15 (2.1)	12 (1.7)	1 (0.1)	-	6 (4.4)

* Five (5) of these cases produced inconclusive results; the table calculations include all cases analysed (n=723).

Central nervous system stimulants (mainly amphetamine-type stimulants) were most frequently detected (n=299, 27.4%), followed by narcotic analgesics (n=208, 19%) and antipyretics (acetaminophen) (n=179, 16.4%) (Table 5). Central nervous system stimulants were predominantly detected in victims of hanging (n=67, 9.3%), drug toxicity (n=31, 4.3%), trauma (n=31, 4.3%) and asphyxia (n=14, 1.9%). Additionally, stimulants and cannabinoids were predominantly detected in deaths caused by burns and/or CO toxicity (both n=11, 64.7% of deaths caused by burns) and gunshot wounds (both n=12, 70.6% of deaths caused by gunshot wound). Narcotic analgesics were frequently detected in victims of drug toxicity (n=42, 5.8%) and less frequently in hanging cases (n=16, 2.2%). Dissociative anaesthetics (ketamine) were found mostly in deaths caused by trauma (n=7, 1.0%).

3.2.3.2. Quantitative analysis

Table 6 illustrates 25 different analytes detected in cases where quantification was possible. Drugs that fell below the lower limit of quantitation (LLOQ) (Appendix A), were not included within the descriptive analysis calculations (e.g., mean, range) (Table 6). Quantitative results were predominantly obtained for methamphetamine (n=41, 38%), acetaminophen (n=40, 37%) and methaqualone (n=35, 32.4%), with higher mean concentrations of the illicit drugs in trauma and suspected SUDA deaths.

Table 5: Total number of times each class of analyte was detected in the different cause of death cases in 723[b] cases analysed.

Drug classes with included drugs [total number of times any drug in drug class was detected] (total number of times analyte was detected)	Cause of death, n (%) (n=number of times any drug in drug class was detected)											
	Asphyxia, 10	**Burns, 17**	**Drowning, 9**	**Gunshot wound, 17**	**Hanging, 69**	**Natural, 24**	**Other[a], 2**	**SUDA, 20**	**SUDI, 15**	**Toxicity, 44**	**Trauma, 40**	**Under investigation, 115**
CNS Stimulants [299]: Cocaine and metabolites* (62), Amphetamine-type stimulants** (233), Methcathinone (4)	14 (1.9)	11 (1.5)	2 (0.3)	12 (1.7)	67 (9.3)	17 (2.3)	2 (0.3)	17 (2.3)	9 (1.2)	31 (4.3)	31 (4.3)	86 (11.9)
Narcotic Analgesics (Opioids/Opiates) [208]: 6-AM (24), Codeine (48), Morphine (49), Hydrocodone (2), Hydromorphone (2), Oxycodone (3), Oxymorphone (2), Fentanyl (6), Tramadol and O-DSMT (70), Methadone (2)	2 (0.3)	3 0.4)	4 (0.5)	11 (1.5)	16 (2.2)	12 (1.7)	-	11 (1.5)	2 (0.3)	42 (5.8)	23 (3.2)	82 (11.3)
Cannabinoids [140]: THC and THC-COOH	6 (0.8)	11 (1.5)	4 (0.5)	12 (1.7)	26 (3.6)	6 (0.8)	-	7 (1.0)	-	5 (0.7)	20 (2.8)	43 (5.9)
Hallucinogens/Dissociative Anaesthetics [17]: Ketamine	-	-	-	2 (0.3)	1 (0.1)	-	-	-	-	2 (0.3)	7 (1.0)	5 (0.7)
Hypnosedatives [166]: Methaqualone (100), Benzodiazepines*** (66)	5 (0.7)	8 (1.1)	2 (0.3)	8 (1.1)	35 (4.8)	10 (1.4)	2 (0.3)	9 (1.2)	5 (0.7)	20 (2.8)	18 (2.5)	44 (6.1)
Antidepressants [26]: Amitriptyline (26)	2 (0.3)	-	-	-	5 (0.7)	1 (0.1)	-	1 (0.1)	-	6 (0.8)	2 (0.3)	9 (1.2)
Antihistamines [58]: Diphenhydramine (58)	1 (0.1)	3 (0.4)	1 (0.1)	5 (0.7)	14 (1.9)	6 (0.8)	-	4 (0.5)	-	2 (0.3)	9 (1.2)	13 (1.8)
Non-narcotic Analgesics/Antipyretics [179]: Acetaminophen (179)	4 (0.5)	5 (0.7)	5 (0.7)	7 (1.0)	24 (3.3)	12 (1.7)	-	14 (1.9)	9 (1.2)	25 (3.5)	18 (2.5)	56 (7.7)
Total number of times analytes were detected (N=1093)	34 (4.7)	41 (5.7)	18 (2.5)	57 (7.9)	188 (26.0)	64 (8.9)	4 (0.5)	63 (8.7)	25 (3.5)	133 (18.4)	128 (17.7)	338 (46.7)
None detected	13 (1.8)	17 (2.3)	14 (1.9)	12 (1.7)	75 (10.4)	9 (8.9)	6 (0.8)	19 (2.6)	41 (5.7)	20 (2.8)	40 (5.5)	111 (15.3)

Acronym: CNS: central nervous system

[a] Combination (blunt force trauma and asphyxia; burns and sharp force trauma)

[b] 723 cases analysed and included in table calculations. Five (5) cases not shown were inconclusive.

*Benzoylecgonine, cocaethylene

**Amphetamine, methamphetamine, MDA, MDMA

***Alprazolam, clobazam, diazepam

Table 5: Post-mortem concentrations of commonly misused drugs in different causes of death in 108 cases (mean, range, standard deviation, number of cases) tested by the Forensic Toxicology Unit.

Total DOA Detected			Drug concentration in mg/L by cause of death, mean (range), SD [n]								
Name, n* (%)	**Mean (range) mg/L**	**# of cases <LLOQ (n)**	**Asphyxia, 2 (1.9)**	**Burns, 2 (1.9)**	**Hang, 3 (2.8)**	**Natural, 5 (4.6)**	**SUDA, 3 (2.8)**	**SUDI, 9 (8.3)**	**Toxicity, 25 (23.1)**	**Trauma, 8 (7.4)**	**Under investigation, 51 (47.2)**
6-Acetylmorphine, 7 (6.5)	0.13 (0.01-0.38)	7	-	-	-	0.13 [1]	-	-	0.16 (0.02-0.38) 0.19 [3]	0.23 [1]	0.03 (0.01-0.53) 0.03 [2]
Acetaminophen, 40 (37)	25.0 (0.11-215)	5	-	-	-	0.92 [1]	6.1 [1]	7.58 (0.13-13) 6.67 [3]	50.8 (0.76-215) 64.4 [15]	0.33 (0.11-0.55) 0.31 [2]	11.6 (0.1-57) 16 [15]
Alprazolam, 12 (11.1)	0.37 (0.01-3.8)	3	-	0.01 [1]	3.8 [1]	0.12 [1]	0.05 [1]	-	0.04 (0.01-0.07) 0.03 [3]	0.01 [1]	0.08 (0.01-0.16) 0.07 [4]
Amitriptyline, 15 (13.9)	3.1 (0.03-19)	-	0.05 [1]	-	-	-	-	-	3.56 (0.04-19) 7.58 [6]	-	3.28 (0.03-12) 5.12 [8]
Amphetamine, 26 (24.1)	0.05 (0.02-0.26)	9	0.02 [1]	-	0.06 [1]	0.03 (0.02-0.03) 0.01 [2]	0.08 [1]	0.04 (0.02-0.07) 0.03 [3]	0.04 (0.03-0.06) 0.01 [3]	0.06 (0.03-0.09) 0.03 [4]	0.07 (0.02-0.26) 0.06 [12]
Benzoylecgonine, 13 (12)	1.3 (0.19-3.2)	-	-	1.0 [1]	-	-	1.7 [1]	-	1.86 (0.49-3.2) 1.1 [5]	0.76 [1]	0.97 (0.19-2.5) 0.94 [5]
Clobazam, 7 (6.5)	0.18 (0.02-0.54)	-	-	-	-	-	-	-	0.17 (0.02-0.4) 0.2 [3]	0.54 [1]	0.07 (0.02-0.1) 0.05 [3]
Cocaethylene, 5 (4.6)	0.12 (0.04-0.3)	2	-	0.04 [1]	-	-	-	-	0.17 (0.08-0.3) 0.12 [3]	-	0.07 [1]
Cocaine, 10 (9.3)	0.77 (0.01-5.3)	2	-	0.03 [1]	-	-	0.03 [1]	-	0.45 (0.02-1.8) 0.76 [5]	-	1.8 (0.01-5.3) 3.05 [3]
Codeine, 20 (18.5)	0.15 (0.01-1.2)	1	-	-	-	0.023 [1]	0.01 [1]	0.06 [1]	0.39 (0.01-1.2) 0.5 [6]	0.05 [1]	0.05 (0.01-0.16) 0.05 [10]
Diazepam, 7 (6.5)	0.11 (0.02-0.36)	1	-	-	-	-	-	-	0.17 (0.02-0.36) 0.14 [4]	0.06 (0.03-0.09) 0.04 [2]	0.09 [1]
Diphenhydramine, 4 (3.7)	0.12 (0.02-0.36)	11	-	-	-	0.3 [1]	0.07 [1]	-	-	0.06 [1]	0.09 [1]
Fentanyl**, 1 (0.9)	1.14	2	-	-	-	-	-	-	-	-	1.4 [1]
Hydrocodone, 1 (0.9)	0.05	-	-	-	-	-	-	-	0.05 [1]	-	-
Ketamine, 2 (1.9)	0.34 (0.07-0.6)	1	-	-	-	-	-	-	0.6 [1]	0.07 [1]	-
MDMA, 1 (0.9)	0.32	-	-	-	-	-	-	-	0.32 [1]	-	-
Methadone, 1 (0.9)	0.28	-	-	-	-	-	-	-	-	-	0.28 [1]
Methamphetamine, 41 (38)	0.25 (0.03-1.1)	2	0.39 [1]	-	0.12 (0.09-0.15) 0.04 [2]	0.20 (0.03-0.37) 0.17 [3]	0.63 [1]	0.07 (0.03-0.18) 0.06 [6]	0.36 (0.04-0.62) 0.24 [4]	0.43 (0.19-0.65) 0.21 [4]	0.32 (0.03-1.1) 0.33 [21]
Methaqualone 35 (32.4)	0.69 (0.04-3.7)	3	-	0.07 [1]	0.96 (0.91-1) 0.06 [2]	1.28 (0.34-1.9) 0.83 [3]	2.17 (0.64-3.7) 2.16 [2]	0.15 (0.07-0.22) 0.06 [4]	0.92 (0.07-1.4) 0.74 [3]	1.13 (0.59-1.79) 0.61 [3]	0.42 (0.04-1.8) 0.44 [19]
Morphine, 22 (20.4)	0.32 (0.01-3)	2	-	-	-	0.27 [1]	0.27 [1]	-	0.80 (0.07-3) 1.25 [5]	0.57 [1]	0.14 (0.01-0.31) 0.11 [14]
O-desmethyltramadol, 10 (9.3)	0.33 (0.01-2.2)	3	-	-	-	-	2.2 [1]	-	0.21 (0.01-0.41) 0.28 [2]	-	0.09 (0.03-0.18) 0.06 [7]
Oxycodone, 2 (1.9)	4.6 (0.07-9.2)	-	-	-	-	-	-	-	0.07 [1]	-	9.2 [1]
Oxymorphone, 1 (0.9)	0.15	1	-	-	-	-	-	-	-	-	0.15 [1]
THC, 5 (4.6)	0.05 (0.02-0.14)	7	-	-	0.03 [1]	-	0.03 (0.02-0.04) 0.02 [2]	-	-	0.08 (0.02-0.14) 0.09 [2]	-
THC-COOH, 17 (15.7)	1.93 (0.02-21)	-	-	-	-	-	21.0 [1]	-	1.26 (0.06-5.2) 2.21 [5]	0.05 [1]	0.54 (0.02-1.4) 0.44 [10]

Acronyms: DOA = drugs of abuse, LLOQ = lower limit of quantification, MDMA = 3,4-Methylenedioxymethamphetamine, THC = Tetrahydrocannabinol, THC-COOH = 11-hydroxy-Δ9-tetrahydrocannabinol

*Number of cases above the LLOQ and included in descriptive analyses.

**Concentration reported in ng/mL.

4. Chapter 4: Discussion

In SA, toxicological investigations other than blood alcohol analyses are not routinely performed in all suspected unnatural and or unexplained death cases, largely due to backlogs at the national laboratories (James, 2015; Liebenberg et al., 2016). While the prevalence of drug misuse (particularly methamphetamine, methaqualone, opioids such as tramadol and heroin, and cannabis) is common in the country, there is currently limited data concerning the role of drugs (other than alcohol) in unnatural and/or unexplained deaths.

The Forensic Toxicology Unit was established within the Western Cape Forensic Pathology Service to assist with and improve toxicological services in death investigations in the province. This study assessed the data from a targeted drugs panel analysis tested on a cohort of suspected unnatural and/or unexplained deaths admitted to Salt River Mortuary in 2022.

4.1. Case characteristics

4.1.1. Demographics

This study included 735 suspected unnatural and/or unexplained death cases for which the targeted analysis was requested. The majority of cases for which specimens were submitted for analyses were males (69.5%) and adults (30-45 years) (38.8%). This was similar to a study conducted at the Pretoria Medico-Legal Laboratory, where 79.2% of cases were males (Liebenberg et al., 2016). These findings aligned with the over-representation of males and middle-aged adults in unnatural deaths in the province, particularly in injury death cases (Western Cape Government Injury Profile, 2016). A similar trend was reported by Auckloo and Davies (2019) in a small cohort of injury-related deaths at Salt River Mortuary (92% males, 38% of cases within 30-49 years).

4.1.2. Cause and alleged manner of death

Following autopsy, pathologists provided a preliminary indication of the suspected manner and cause of death on the toxicology request form. At the time of this study, cases were indicated to be under investigation for both manner (46.8%) and cause (29.5%) of death. This number of 'under investigation' cases was a drawback, as the pathologists' final conclusion on the role of drug and/or chemical toxicity in these deaths was not available.

The next most frequently recorded alleged manner of death was suicide (28.7%), most of which were hangings (63.5%). This was similar to the Western Cape Injury Profile report of 2016, which reported that 60% of the suicide deaths were as a result of hanging (Western Cape Injury Profile, 2016). During medicolegal investigations of suicide deaths, toxicological analysis may provide relevant and useful information pertaining to the individuals mental state around death or providing insight into prior drug misuse. Drugs and alcohol are reported in studies on suicide (San Nicolas & Lemos, 2015), and suicidal thoughts and behaviours may be precipitated by the use of drugs and/or alcohol (Collados-Ros et al., 2022).

The current study included numerous infant deaths (14.7%), with the majority of these cases noted as SUDI (47%) or under investigation (40%). Various studies have highlighted the prevalence of SUDI cases in Cape Town, SA. Nine percent (n=1,608) of cases admitted to Salt River Mortuary between 2013 and 2017 were infants younger than 1 year old, of which 75% (n=1,199) were further classified as SUDI (Heathfield et al., 2020). Similarly, 9% (n=1,271) of cases admitted to Tygerberg Mortuary between 2005 and 2009 were categorised as SUDI, with toxicological analysis requested only in 0.5% of cases (du Toit-Prinsloo et al., 2013). Furthermore, the Western Cape injury profile (2016) reported that 8% of all deaths amongst children younger than 5 years were SUDI.

There is no standardised protocol for the investigation of SUDI cases in SA (du Toit-Prinsloo et al., 2013; Heathfield et al., 2020), and toxicological investigations may be necessary to exclude other contributing factors such as toxicity, given how vulnerable infants are to drugs, toxicants and chemicals (Dolinak, 2013). Testing for commonly misused drugs may provide useful information pertaining to the case at hand, such as circumstances surrounding death including child abuse and neglect. In addition, drug analyses following SUDI are not routinely performed in SA, despite the relevance of a possible toxic exposure, which could cause death or exacerbate natural deaths. This current study should serve as a stepping stone towards future research and implementation of routinely analysing these cases for the presence of drug(s) and other toxicants.

In this study, drugs such as stimulants and hypno-sedatives were also found in cases where cause of death was later determined as natural. Substance use, particularly involving stimulants like methamphetamine and hypno-sedatives such as methaqualone, can impact outcomes in natural deaths (Pilgrim et al., 2009), although drug testing is often not requested, even in cases where these

substances could have played a major role. This is particularly important to consider as there are numerous instances where deaths initially suspected to be unnatural are later deemed natural, both in infants and adults. Stimulants increase central nervous system activity, resulting in elevated heart rate and blood pressure, which can cause cardiovascular complications such as myocardial infarction and the rupture of saccular aneurysms (Westover et al., 2008; Pilgrim et al., 2009; Sinha et al., 2016). They can also weaken the immune system, reducing the body's ability to combat infections (Thennakoon, 2015), which can contribute to natural disease. Similarly, hypno-sedatives depress the central nervous and respiratory systems, leading to respiratory depression, impaired reflexes, which may contribute to deaths from respiratory failure or other natural complications (Rassool & Winnington, 2006; Phelps & Hassed, 2012). Given the prevalence of such substances, it is recommended to conduct a routine drug screening not only in "injury-related" deaths but also in those deemed unnatural and/or unexplained at admission to the mortuary.

4.2. Forensic Toxicology Unit toxicological results

A high rate of positive drug detections was observed (52.8% of the 723 cases analysed). In addition, 29 different analytes were detected, (only cannabidiol (CBD) and buprenorphine were not detected), indicating the applicability of the panel. Acetaminophen was the most commonly detected drug, followed by methamphetamine, amphetamine, methaqualone and THC-COOH, all recognised to be frequently misused drugs in SA (Liebenberg et al., 2016; Auckloo & Davies, 2019; Benedict et al., 2019; Els et al., 2020).

Acetaminophen (also known as paracetamol) is a non-opioid analgesic and antipyretic that is commonly used worldwide to treat fever and mild to moderate pain (Bunchorntavakul & Reddy, 2013). Acetaminophen remains the most commonly used drug in deliberate self-harm in the country (Ani et al., 2017; Benedict et al., 2019). The high prevalence of this drug in this study is an indication of its accessibility as an over-the-counter product in SA. The drug was frequently detected in under investigation, toxicity, or unexpected deaths. Concentrations suggest that it may have been an incidental finding in many cases, however, some toxicity death cases may have been due to acetaminophen (with or without other drugs) (e.g., the highest concentration reported in the study was 215 mg/L). In cases where acetaminophen toxicity contributed to the death, it is typically acute liver failure (hepatotoxicity) as a result of overdose (Nourjah et al., 2006; Lee, 2008;

Hodgman & Garrard, 2012). However, this current study did not assess any specific pathology associated with the deaths.

Methamphetamine (locally known as 'tik' or crystal meth) and its primary metabolite, amphetamine, are extremely potent and euphoric central nervous system stimulants that are frequently associated with violent and aggressive behaviour in its users (Chen et al., 2016). Previous studies have also reported on the high prevalence of methamphetamine (and amphetamine) in SA, particularly in Cape Town (Plüddemann et al., 2013; Liebenberg et al., 2016). In 2022, methamphetamine was reported to be the most frequently used drug in the Western Cape region, making it the second most used drug in SA, following cannabis (SACENDU, 2022). In the medico-legal context, it has been linked to higher rates of violence (Watt et al., 2015) and suicide fatalities (Darke et al., 2009,). The high availability of methamphetamine in communities might be explained by its cheaper prices and ease of making (as it can be made from commonly available ingredients) (Hobkirk et al., 2016).

Methamphetamine was detected in 127 cases (33% of positive cases), including 15 child deaths (<15 years old), and at least 9 infant deaths. These are important findings warranting further exploration into the contextual factors surrounding methamphetamine exposure in children. Some of these cases might have been due to environmental exposure, such as inhaling secondary smoke from parents, other close family members or caretakers. Accidental ingestion of drugs or chemicals that are within a young child's reach, or ingestion through breast milk from the mother, are also common (Dolinak, 2013). One such case was investigated by Vasudevan (2019), where a mother using methamphetamine breastfed her six-week-old daughter, which then led to the infant dying of methamphetamine toxicity. Without toxicological investigations, these cases would become difficult to solve and further precautions and interventions would not easily be taken. Although the mere detection of these substances does not indicate that it caused or contributed to death, it is nonetheless necessary to take all potential causes into account, particularly given how vulnerable infants are, and is contextually important as it may point to neglect and/or abuse.

Methamphetamine concentrations overlapped between traumatic deaths and those due to possible toxicity or that were still under investigation. Deaths due to trauma (0.43 mg/L), asphyxia (0.39 mg/L), SUDA (0.63 mg/L) and drug toxicity (0.36 mg/L) had mean methamphetamine

concentrations in the reported 'clinical' toxic range (0.2 to 1 mg/L) (Schulz et al., 2020). However, interpreting these within a post-mortem context is more complex. It is recommended to build up quantitative control data from cases where methamphetamine is detected but not deemed causal to death, to improve interpretation in toxicity and other cases.

Methaqualone was the most frequently detected substance in a post-mortem study by Stewart et al. (2010) conducted in Gauteng, SA, followed by opiates, cocaine and amphetamine, which correlate relatively well with the findings of this study. Methaqualone together with diphenhydramine is locally known as 'mandrax' and is a synthetic hypno-sedative substance that depresses the central nervous system (Rossiter, 2017). As such, it was historically prescribed to treat anxiety and insomnia. Mandrax is often co-ingested with other drugs such as cannabis (known as 'bottleneck' or 'white pipe'), which produces a greater rush feeling and lasts for a long period (Rock & Moore, 1976; Wechsberg et al., 2012). Methaqualone may often be used with other drugs (Vitale & van de Mheen, 2006), such as methamphetamine (or ecstasy) to ease the methamphetamine withdrawal (Chelin, 2021). The combined detection of methamphetamine, methaqualone, and diphenhydramine was frequent in this study, and reported previously in Salt River Mortuary deaths (Auckloo & Davies, 2019) as well as unnatural deaths in Pretoria Liebenberg et al. (2016).

Despite the very high prevalence of methaqualone in SA (and other countries including China and India), its use has been declining worldwide (Brownfield, 2010; Liebenberg et al., 2016; UNODC, 2021, Inger et al., 2023). This is mainly due to strict regulations regarding the possession and production of this drug around the world, more especially in the United States (Herzberg, 2011; DEA, 2018). As of 2021, methaqualone is still being produced in clandestine laboratories in SA, which continues to be a serious law enforcement problem (Chelin, 2021; Inger et al., 2023).

The high proportion of cases in this study in which THC and its metabolite (THC-COOH) were detected was expected since cannabis is known to be one of the most frequently used drugs in SA and worldwide (Nel, 2013; Liebenberg et al., 2016; CDA, 2021/22; UNODC, 2022). It was recently decriminalised for personal use in SA (Lubaale & Mavundla, 2019). While both THC and THC-COOH were quantified, it may be suggested that this is not necessary in post-mortem cases,

particularly with the challenges in interpretation post-mortem due to factors such as redistribution (Tascon et al., 2023).

Benzodiazepines, particularly alprazolam and diazepam were frequently detected in the cohort. Alprazolam is a commonly prescribed benzodiazepine in SA for anxiety (Ait-Daoud et al., 2018), and diazepam is also utilised frequently in hospital (such as to treat seizures in overdoses if an individual was admitted to hospital before death) (Pok et al., 2010; Faulkner, 2015). Alprazolam was detected in 29 of the cases included in this study and quantified in 12 cases, with one case classified as 'hanging' having a mean blood concentration of 3.8 mg/L. Previous studies have reported on the alprazolam postmortem blood concentrations (Wolf et al., 2005; Jones & Holmgre, 2013; Jönsson et al., 2014; Feola et al., 2023) and these were relatively similar in comparison to the findings of this current study. Michaud et al. (1999) reported on the fatal blood concentrations of alprazolam which were between 0.12 and 0.39 mg/L. Only a few deaths (overdoses) due to alprazolam toxicity alone have been documented (Jenkins et al., 1997; Michaud et al., 1999; Feola et al., 2023), in contrast to the majority of deaths resulting from co-ingestion with other drugs (such as alcohol and opioids) and resulting in adverse drug-drug interactions.

Drugs such as amitriptyline were frequently detected in females. Amitriptyline is an antidepressant medication that can cause cardiac death at high doses (Ray et al., 2004). The drug is frequently detected in female suicides (Sheehan et al., 2013). Previous studies found that amitriptyline is typically ingested by young females (Aslan et al., 2011; Güloğlu et al., 2011). Concentrations of amitriptyline in this study were very high and frequently involved in 'under investigation' cases (averaging >3 mg/L), indicating that it was likely involved (with or without other drugs) in several death cases. It was also detected in a SUDI case, suggesting possible exposure through breast milk (Davanzo et al., 2014, Amundsen et al., 2015). This highlights the importance of amitriptyline inclusion in the panel, given the frequency of use in SA (Coetzee et al., 2020), and indicates the necessity of expanding the testing to other key anti-depressant drugs.

6-Acetylmorphine (6-AM), a unique heroin metabolite (Yu et al., 2015), was detected in 24 cases, and quantitatively analysed in 14 (seven of which were below the lower limit of quantitation). The detection of this analyte, together with morphine (detected in 49 cases), is suggestive of heroin administration and was typically identified in toxicity cases. There was

however, one case deemed to be a natural death by the pathologist, where the detection of this analyte was likely relevant for further discussion or cause of death review. Other opioids such as methadone, oxycodone and fentanyl were infrequent in number. This is in stark contrast to the opioid crisis in the United States (Brown & Sloan, 2017; Kertesz & Gordon, 2019), in which most drug-related deaths are typically related to fentanyl and its analogues. Similarly, the UNODC report of 2022 showed that opioids (mainly heroin and fentanyl) have been the primary cause of drug-related mortality in the last few decades, with overdoses accounting for the majority of these deaths (UNODC, 2022). This study did however indicate that opioids (mostly heroin, codeine and tramadol) were frequent in cases classified as 'drug toxicity', indicating the relevance of monitoring opioids in unnatural and/or unexplained death.

Ketamine was most commonly found in victims of trauma, despite the very low number of cases. This seems prudent given the drugs ability to alter the user perception, emotions and thoughts (Corkery et al., 2021). It is, however, possible that many of these cases were individuals who died from their traumatic injuries in hospital, in which ketamine was administered as an anaesthetic or analgesic following severe injuries.

Other drugs of abuse such as MDMA, methylenedioxyamphetamine (MDA), and methcathinone were detected less frequently in the current study. Although drugs such as MDMA and methcathinone (locally known as Khat) are common in SA (Plüddemann et al., 2008; Pelterz et al., 2010), the low incidence of these drugs might be an indication of the differences in drugs commonly misused across South African provinces. For instance, methcathinone was reportedly common in the Gauteng province, while ecstasy is frequent in the Kwazulu Natal and Northen regions of SA (CDA, 2021/22). Infrequent or not detected analytes (e.g., CBD and buprenorphine) may be due to infrequent use. In addition, long storage time before analysis may have affected the integrity of the sample, thereby, making it difficult to detect certain analytes (da Cunha et al., 2018; Aldubayyan et al., 2021).

4.3. Forensic Chemistry Laboratory analytical results

Pathologists submitted specimens for BAC analyses to the National Forensic Chemistry Laboratory in the majority of included cases (73.6%). Very few cases (28.0%) were submitted to the National laboratory for additional drug testing, likely due to the delays in testing (highlighted by outstanding results from the Forensic Chemistry Laboratory for both BAC (26%) and toxicology (89%) testing). These delays in National toxicology are a recognised problem within the country, and which can result in delays in cause of death determination and case conclusion.

The few cases with positive toxicological results (n=15) from the Forensic Chemistry Laboratory were similar to the findings of the Forensic Toxicology Unit, with acetaminophen and methamphetamine most commonly detected. These findings highlight the frequency of these drugs in unnatural deaths in the Western Cape region, more particularly in Cape Town.

4.3.1. Blood alcohol analysis

Alcohol-related deaths are a major public health concern in SA (Rataemane S & Rataemane L, 2006; Plüddemann et al., 2010, Matzopoulos et al., 2014), particularly as they relate to injury deaths (Bachan et al., 2023). Despite the low number of positive BACs (n=138, 25.6%) reported in this study, it must be considered that approximately the same number of cases still had outstanding results. The mean BAC (0.15 g/100 mL) correlated with a previous study conducted at Salt River Mortuary investigating violent fatalities (0.18 g/100 mL) (Auckloo & Davies, 2019). The majority of the positive BAC cases in this current study in which ethanol was detected had BACs higher than the legal driving limit in SA (> 0.05 g/100 mL) (n=103, 75%) (National Road Traffic Act (Act 93 of 1996), and alcohol intoxication around the time of death should be considered (particularly if also positive for psychoactive drugs).

In addition, one or more drugs (especially methamphetamine and THC-COOH) were detected in half of the cases that had positive BACs, with males and middle-aged adults accounting for the majority of these cases. Combining psychoactive drug use with alcohol, can exacerbate drug effects, as demonstrated when alcohol is consumed with cocaine, resulting in the formation of cocaethylene (Wetli, 2007). In this current study, 79% of cases in which cocaethylene was detected also tested positive for ethanol suggesting recent use of cocaine and alcohol before death (and before elimination of ethanol or cocaethylene). In a similar study, Skoop (2010) highlighted the

danger of ethanol interacting with other drugs (especially sedative hypnotics, given the central nervous system depressant effects of alcohol).

The largest number of alcohol-positive cases were hanging-related suicides, which agrees with a study by Jones et al. (2013). The impulsivity and loss of inhibitions brought on by severe drinking may precipitate suicidal thoughts and behaviors (Pompili et al., 2010). This highlights the necessity for implementation and interventions to address and monitor the danger and fatal consequences of alcohol misuse across our communities, more especially among individuals who are battling with mental illness. In addition, ethanol was predominant in decedents of CO toxicity and/or burns (n=16), trauma (n=15) and asphyxia (n=10). Ethanol is a central nervous system depressant and behaviour impairing drug, and as such, it can slow down brain activities (both cognitive and physical capacities), thereby, leading to decreased reaction time, drowsiness and decreased inhibition (Schuckit, 2011; Pompili et al., 2010; Mukherjee, 2013), and thus possibly resulting in delayed reaction to events (such as the inability to escape from fire).

In this study, one case with a natural cause of death, also had a BAC of 0.36 g/100 mL, which suggests that this case and other similar cases might need to be re-examined after toxicology results become available. Fatal ethanol concentrations (BAC>0.40 g/100 mL) were detected in two cases (0.50 and 0.54 g/100 ml) that were still under investigation at the time of the study.

4.4. Limitations and recommendations

Approximately 89% of the toxicology results from the National Forensic Chemistry Laboratory were outstanding at the time of the study. This highlights the historic backlogs in processing toxicology casework faced by the National laboratory. While the Forensic Toxicology Unit only presented a limited analytical panel, all qualitative and quantitative results were available to pathologists at the study conclusion, demonstrating that the laboratory has improved the availability of drug data in unnatural and/or unexplained deaths in the province.

This study only investigated unnatural and/or unexplained deaths admitted to Salt River Mortuary (and a few cases from Tygerberg Mortuary) for which toxicological analyses were requested from the Forensic Toxicology Unit. The results from this study do not necessarily represent the overall drug findings in the unnatural and/or unexplained death population in Cape Town or the Western Cape. In addition, the panel of testing was limited, and further comprehensive testing using broad drug and chemical screening is required to further ascertain the role of other drugs, pesticides and chemicals in death in the province. In addition, the expansion of these methods to other specimens such as vitreous humor, as well as specimens that may represent chronic drug use (such as hair or nails), may provide further information on the role of drugs in death in the community. Nevertheless, this study does provide important quantitative and validated drug data in a cohort of deceased individuals in Western Cape and provides a framework for further studies in this area in the country.

The causes and manners of death were still under investigation for many cases at the time of the study's conclusion, resulting in the loss of valuable data. In future, it may be valuable to conduct interviews with pathologists on the role these tests play in their cause of death determination, and how they utilised the results in their conclusions, possibly assisting in strengthening the full medico-legal workflow.

While this study indicated the detection of drugs within the post-mortem toxicology context, it does not indicate that those analytes specifically were the culprits in causing toxicity to the deceased (this is true also for ‘drug toxicity’ cases). For instance, some cases may have been clinically given drugs such as diazepam and morphine in the hospital if they were admitted for a drug overdose and were seized or needed pain relief. In addition, given the few quantitative analyses performed and

not having final cause of death after the pathologist seen the toxicological results, it was not possible to state the role of the detected drugs in contributing to or causing death.

Quantitative analysis was only performed by the Forensic Toxicology Unit in just over a quarter of the positive cases, largely for cases that were under investigation and did not have a direct cause of death noted at autopsy (e.g. blunt force trauma or hanging). Quantitation in all cases will assist in building up quantitative data and drug concentrations in different types of death to improve local interpretation, however, this has to be weighed up against costs and workload, together with pathologist requirements in a particular case.

Other factors such as decomposition, delay in the analysis of samples and post-mortem redistribution might have affected the integrity of the specimens, leading to a lower likelihood of detecting some of the potential drugs of abuse present in the deceased body. It is advised to sample specimens from different sites (e.g., cardiac and peripheral blood, vitreous humor) and analyse them timeously, to increase the chances of detecting the analytes of interest, and improve the reliability associated with interpreting quantitative results.

Chapter 5: Conclusion

This study reports on post-mortem toxicological data obtained from a validated qualitative and quantitative LC-MS/MS panel for 31 commonly misused drugs in Cape Town, SA. Scheduled recreational drugs including methamphetamine, amphetamine and methaqualone (together with diphenhydramine in the form of 'mandrax') were the most frequently detected drugs in a cohort of suspected unnatural and/or unexplained deaths in Cape Town in 2022 (with drugs being detected in over 50% of the cases). Cases were positive for most drugs in the panel, indicating its relevance in local toxicological testing. In addition, this study highlighted the detection of illicit and impairing drugs in sudden infant death, and in injury deaths such as hangings (where judgement may be impaired). This data supports the need for routine drug testing in various injury-related deaths (in addition to suspected toxicity deaths). It provides a framework for further investigation into drugs in unnatural and/or unexplained death and provides validated quantitative data that may be built on to improve interpretations of concentrations of drugs within the local post-mortem population.

References

Ait-Daoud, N., Hamby, A.S., Sharma, S. & Blevins, D. 2018. A review of alprazolam use, misuse, and withdrawal. *Journal of Addiction Medicine*. *12*(1):4.

Aldubayyan, A.A., Castrignanò, E., Elliott, S. & Abbate, V. 2021. Stability of synthetic cathinones in clinical and forensic toxicological analysis—where are we now?. *Drug testing and analysis*. *13*(1):44-68.

Amundsen, S., Nordeng, H., Nezvalová-Henriksen, K., Stovner, L.J. & Spigset, O. 2015. Pharmacological treatment of migraine during pregnancy and breastfeeding. *Nature Reviews Neurology*. 11(4):209-219.

Ani, J.O., Ross, A.J. & Campbel, L.M. 2017. A review of patients presenting to accident and emergency department with deliberate self-harm, KwaZulu-Natal, South Africa. *African Journal of Primary Health Care and Family Medicine*. *9*(1):1-7.

Aslan, Ş., Emet, M., Cakir, Z., AKÖZ, A. & GÜR, S.T.A. 2011. Suicide attempts with amitriptyline in adults: a prospective, demographic, clinical study. *Turkish Journal of Medical Sciences*. 41(2):243-249.

Auckloo, M.B.K.M. & Davies, B.B. 2019. Post-mortem toxicology in violent fatalities in Cape Town, South Africa: A preliminary investigation. *Journal of Forensic and Legal Medicine*. 63:18–25.

Bachan, V., Molefe, I. & Davies, B. 2023. Investigating blood alcohol concentrations in injury-related deaths before and during the COVID-19 national lockdown in Western Cape, South Africa: a cross-sectional retrospective review. *South African Medical Journal.* 50-56.

Bailey, M.J., Randall, E.C., Costa, C., Salter, T.L., Race, A.M., De Puit, M., Koeberg, M., Baumert, M. & Bunch, J. 2016. Analysis of urine, oral fluid and fingerprints by liquid extraction surface analysis coupled to high resolution MS and MS/MS–opportunities for forensic and biomedical science. *Analytical Methods*. *8*(16):3373-3382.

Benedict, M.O.A., Van Loggerenberg, C.J. & Steinberga, W.J., 2019. The profile of deliberate self-poisoning cases presenting at the emergency department of Pelonomi Regional Hospital, Bloemfontein. *South African Family Practice*. *61*(1):11-17.

Brown Jr, R.E. & Sloan, P.A. 2017. The opioid crisis in the United States: Chronic pain physicians are the answer, not the cause. *Anesthesia & Analgesia*. *125*(5):1432-1434.

Brownfield, W. 2010. International Narcotics Control Strategy Report. Drug and Chemical Control. Reports – South Africa. *Washington, DC: United States Department of State, Bureau for International Narcotics and Law Enforcement Affairs*. 1:554-559

Bucello, C., Degenhardt, L., Calabria, B., et al. 2010. What do we know about the extent of cocaine use and dependence? Results of a global systematic review—NDARC technical report No 308. Sydney: *National Drug and Alcohol Research Centre, University of NSW*.

Bunchorntavakul, C. & Reddy, K.R. 2013. Acetaminophen-related hepatotoxicity. *Clinics in liver disease. 17*(4):587-607.

Calabria, B., Degenhardt, L., Nelson, P., et al. 2010. What do we know about the extent of cannabis use and dependence? Results of a global systematic review—NDARC technical report No 307. Sydney: National *Drug and Alcohol Research Centre, University of NSW*.

Carrier, N. & Klantschnig, G. 2012. *Africa and the War on Drugs.* Bloomsbury Publishing.

Central Drug Authority (CDA). 2021/22. Annual Report 2021/22. *Pretoria: Department of Social Development, Republic of South Africa*. Available: http://www.dsd.gov.za/cda/index.php?option=com_docman&task=doc_download&gid=53&Itemid=116 [2023, June 01].

Chelin, R. 2021. The white pipe keeps burning - ISS Africa. Institute for Security Studies Today. Available: https://issafrica.org/iss-today/the-whitepipe-keeps-burning [2023, December 14].

Chen, L.Y., Crum, R.M., Strain, E.C., Alexander, G.C., Kaufmann, C. & Mojtabai, R. 2016. Prescriptions, nonmedical use, and emergency department visits involving prescription stimulants. *The Journal of Clinical Psychiatry*. *77*(3):15331.

Coetzee, R., Johnson, Y., van Niekerk, J. & Namane, M. 2020. Amitriptyline prescribing in public sector healthcare facilities in the Western Cape, South Africa. *PLoS One. 15*(4):e0231675.

Collados-Ros, A., Torres-Sánchez, C., Pérez-Cárceles, M.D., Luna, A. & Legaz, I. 2022. Suicidal behavior and its relationship with postmortem forensic toxicological findings. *Toxics. 10*(6):319.

Corkery, J.M., Hung, W.C., Claridge, H., Goodair, C., Copeland, C.S. & Schifano, F. 2021. Recreational ketamine-related deaths notified to the National Programme on Substance Abuse Deaths, England, 1997–2019. *Journal of Psychopharmacology*. 35(11):1324-1348.

da Cunha, K.F., Eberlin, M.N. & Costa, J.L. 2018. Long-term stability of synthetic cathinones in dried blood spots and whole blood samples: a comparative study. *Forensic Toxicology*. *36*:424-434.

Darke, S. 2010. The toxicology of homicide offenders and victims: a review. *Drug Alcohol Review*. 29(2):202–215.

Darke, S., Degenhardt, L. & Mattick, R. 2006. *Mortality Amongst Illicit Drug Users: Epidemiology, Causes and Intervention*. Cambridge: Cambridge University Press.

Darke, S., Duflou, J. & Torok, M. 2009. Toxicology and circumstances of completed suicide by means other than overdose. *Journal of Forensic Science*. 54(2):490–494.

Davanzo, R., Bua, J., Paloni, G. & Facchina, G. 2014. Breastfeeding and migraine drugs. European journal of clinical pharmacology. 70:1313-1324.

Degenhardt, L. & Hall, W. 2012. Extent of illicit drug use and dependence, and their contribution to the global burden of disease. *The Lancet. 379*(9810):55-70.

Degenhardt, L., Bucello, C., Calabria, B., et al. 2011. What data are available on the extent of illicit drug use and dependence globally? Results of four systematic reviews. *Drug Alcohol Depend*. 117(2-3):85-101.

Degenhardt, L., Chiu, W.T., Sampson, N., Kessler, R.C., Anthony, J.C., Angermeyer, M., Bruffaerts, R., De Girolamo, G., Gureje, O., Huang, Y. & Karam, A. 2008. Toward a global view of alcohol, tobacco,

cannabis, and cocaine use: findings from the WHO World Mental Health Surveys. *PLoS Medicine*. 5(7):141.

Dolinak, D. 2013. Forensic Toxicology: *A Physiologic Perspective.* First ed. Calgary: Academic Forensic Pathology International.

Donaldson, A.E., Larsen, G.Y., Fullerton-Gleason, L. & Olson, L.M. 2006. Classifying undetermined poisoning deaths. *Injury Prevention*. *12*(5):338-343.

Drug Enforcement Administration (DEA). 2018. Our History (1980−1985); United States Drug Enforcement Administration.

Drummer, O.H. & Gerostamoulos, D. 2013. Forensic drug analysis. *Future Science Ltd.*

Drummer, O.H. & Gerostamoulos, J. 2002. Postmortem drug analysis: analytical and toxicological aspects. *Therapeutic Drug Monitoring. 24*(2):199-209.

Drummer, O.H. 2004. Postmortem toxicology of drugs of abuse. *Forensic Science International*. 142(2–3):101–113. DOI: 10.1016/j.forsciint.2004.02.013.

Drummer, O.H. 2007. Requirements for bioanalytical procedures in postmortem toxicology. *Analytical and Bioanalytical Chemistry.* 388(7):1495–1503. DOI: 10.1007/s00216-007-1238-7.

Drummer, O.H. 2013. Post-mortem toxicology in the elderly. *Forensic Science, Medicine, and Pathology. 9:*258-259.

du Toit-Prinsloo, L., Dempers, J., Verster, J., Hattingh, C., Nel, H., Brandt, V.D., Jordaan, J. & Saayman, G. 2013. Toward a standardized investigation protocol in sudden unexpected deaths in infancy in South Africa: a multicenter study of medico-legal investigation procedures and outcomes. *Forensic science, Medicine, and Pathology*. *9*:344-350.

Els, J.R., Taljaard, L. & Strover, B. 2020. Delayed presentation of paracetamol overdose. *African Journal of Emergency Medicine*. *10*(3):170-172.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). 2022. E*uropean Drug Report: Trends and Development*. Available: European Drug Report 2022: Trends and Developments | www.emcdda.europa.eu [2023, Oct 10].

Faulkner, M.A. 2015. Comprehensive overview: efficacy, tolerability, and cost-effectiveness of clobazam in Lennox–Gastaut syndrome. *Therapeutics and Clinical Risk Management.* 905-914.

Feola, A., Carfora, A., Mascolo, P., Petrella, R., Della Pietra, B. & Campobasso, C.P. 2023. Alprazolam-associated death: suicide or unintentional overdose?. *Australian Journal of Forensic Sciences.* 55(1):3-11.

Finkelstein, Y., Goel, G., Hutson, J.R., Armstrong, J., Baum, C.R., Wax, P., Brent, J. & Toxicology Investigators Consortium. 2017. Drug misuse in adolescents presenting to the emergency department. *Pediatric Emergency Care. 33*(7):451-456.

Güloğlu, C., Orak, M., Üstündağ, M. & Altuncı, Y.A. 2011. Analysis of amitriptyline overdose in emergency medicine. *Emergency Medicine Journal.* 28(4):296-299.

Heathfield, L.J., Martin, L.J. & Ramesar, R. 2020. A 5-year retrospective analysis of infant death at Salt River Mortuary, Cape Town. *South African Journal of Child Health*. *14*(3):148-154.

Herzberg, D. 2011. Blockbusters and controlled substances: Miltown, Quaalude, and consumer demand for drugs in postwar America. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*. *42*(4):415-426.

Hill, S.L. & Thomas, S.H. 2020. Drug misuse. *Medicine. 48*(3):176-181.

Hobkirk, A.L., Watt, M.H., Myers, B., Skinner, D. & Meade, C.S. 2016. A qualitative study of methamphetamine initiation in Cape Town, South Africa. *International Journal of Drug Policy*. *30*:99-106.

Inger, J.A., Mihan, E.R., Kolli, J.U., Lindsley, C.W. & Bender, A.M. 2023. DARK Classics in chemical neuroscience: methaqualone. *ACS Chemical Neuroscience*. *14*(3):340-350.

James, W. 2015. The state of Forensic Chemistry Laboratories in South Africa. Available: The state of Forensic Chemistry Laboratories in SA - Wilmot James - POLITICS | Politicsweb [2023, Nov 24].

Jenkins, A.J., Levine, B., Locke, J.L. & Smialek, J.E. 1997. A fatality due to alprazolam intoxication. *Journal of Analytical Toxicology*. 21(3):218-220.

Jones, A.W., & Holmgren, A. 2013. Concentrations of alprazolam in blood from impaired drivers and forensic autopsies were not much different but showed a high prevalence of co-ingested illicit drugs. *Journal of Psychopharmacology. 27(3):276-281.*

Jones, A.W., Holmgren, A. & Ahlner, J. 2013. Toxicology findings in suicides: concentrations of ethanol and other drugs in femoral blood in victims of hanging and poisoning in relation to age and gender of the deceased. *Journal of Forensic and Legal Medicine.* 20(7):842-847.

Jönsson, A.K., Söderberg, C., Espnes, K.A., Ahlner, J., Eriksson, A., Reis, M. & Druid, H. 2014. Sedative and hypnotic drugs—fatal and non-fatal reference blood concentrations. *Forensic Science International*. *236*:138-145.

Kerrigan, S. 2013. Sampling, storage and stability. *Clarke's Analytical Forensic Toxicology*. *2*:335-347.

Kertesz, S.G. & Gordon, A.J. 2019. A crisis of opioids and the limits of prescription control: United States. *Addiction*. *114*(1):169-180.

Lehohla, P.J. 2014. *Mid-year Population Estimates 2014 – Statistical Release (P0302)*. Pretoria: Statistics South Africa, 2014. Available: http://www.statssa.gov.za/publications/P0302/P03022014.pdf [2023, March 01].

Liebenberg, J., du Toit-Prinsloo, L., Saayman, G. & Steenkamp, V. 2019. Drugged driving in South Africa. *South African Crime Quarterly. 67*(1):7-18.

Liebenberg, J., du Toit-Prinsloo, L., Steenkamp, V. & Saayman, G. 2016. Fatalities involving illicit drug use in Pretoria, South Africa, for the period 2003 - 2012. *South African Medical Journal*. 106(10):1051–1055. DOI: 10.7196/SAMJ.2016.v106i10.11105.

Lubaale, E.C. & Mavundla, S.D. 2019. Decriminalisation of cannabis for personal use in South Africa. *African Human Rights Law Journal*. *19*(2):819-842.

Matzopoulos, R.G., Truen, S., Bowman, B. & Corrigall, J. 2014. The cost of harmful alcohol use in South Africa. *South African Medical Journal. 104*(2):127-132.

Michaud, K., Augsburger, M., Romain, N., Giroud, C. & Mangin, P. 1999. Fatal overdose of tramadol and alprazolam. *Forensic Science International*. *105*(3):185-189.

Moriya, F. & Hashimoto, Y. 1996. Postmortem stability of cocaine and cocaethylene in blood and tissues of humans and rabbits. *Journal of Forensic Sciences*. *41*(4):612-616.

Mukherjee, S. 2013. Alcoholism and its effects on the central nervous system. *Current neurovascular research*. *10*(3):256-262.

National Health Laboratory Services (NHLS). 2022. *Annual report 2022-2023*. Available: 241023-NHLS-Annual_Report.pdf [2023, Dec 7].

Nel, E. 2013. Monitoring Alcohol and Drug Abuse Treatment Admissions in South Africa – Data for Gauteng. Phase 33. South African Community Epidemiology Network on Drug Use (SACENDU). *Cape Town: Alcohol, Tobacco and Other Drug Research Unit, Medical Research Council*. 14-27.

Nelson P, McLaren J, Degenhardt L, et al. 2010. What do we know about the extent of heroin and other opioid use and dependence? Results of a global systematic review—NDARC technical report No. 309. Sydney: *National Drug and Alcohol Research Centre, University of NSW*.

Nourjah, P., Ahmad, S.R., Karwoski, C. & Willy, M. 2006. Estimates of acetaminophen (paracetomal)-associated overdoses in the United States. *Pharmacoepidemiology and Drug Safety. 15*(6):398-405.

Pan, M., Wang, X., Zhao, Y., Liu, W. & Xiang, P. 2019. A retrospective analysis of data from forensic toxicology at the Academy of Forensic Science in 2017. *Forensic Science International. 298:*39-47.

Peacock, A., Leung, J., Larney, S., Colledge, S., Hickman, M., Rehm, J., Giovino, G.A., West, R., Hall, W., Griffiths, P. & Ali, R. 2018. Global statistics on alcohol, tobacco and illicit drug use: 2017 status report. *Addiction*. *113*(10):1905-1926.

Peltzer, K. & Phaswana-Mafuya, N. 2018. Drug use among youth and adults in a population-based survey in South Africa. *South African Journal of Psychiatry*. *24*(1):1-6.

Peltzer, K., Ramlagan, S., Johnson, B.D. & Phaswana-Mafuya, N. 2010. Illicit drug use and treatment in South Africa: a review. *Substance Use & Misuse. 45*(13):2221-2243.

Peters, F.T., Drummer, O.H. & Musshoff, F. 2007. Validation of new methods. *Forensic Science International.* 165(2–3):216–224.

Phelps, K. & Hassed, C. 2012. *Geriatric Medicine: General Practice: The Integrative Approach Series*. Elsevier Health Sciences.

Pilgrim, J.L., Gerostamoulos, D., Drummer, O.H. & Bollmann, M. 2009. Involvement of amphetamines in sudden and unexpected death. *Journal of Forensic Sciences*, *54*(2):478-485.

Plüddemann, A., Dada, S., Bhana, A., et al. 2010. Monitoring alcohol & drug abuse trends in South Africa (july 1996-June 2010). S*ACENDU Reasearch*.13(2)

Plüddemann, A., Dada, S., Parry, C., Bhana, A., Bachoo, S., Perreira, T., Nel, E., Mncwabe, T., Gerber, W. & Freytag, K. 2008. Monitoring alcohol and drug abuse trends in South Africa. *Drug Alcohol Review. 2*(27):185-189.

Plüddemann, A., Dada, S., Parry, C.D., Kader, R., Parker, J.S., Temmingh, H., Van Heerden, S., De Clercq, C. & Lewis, I. 2013. Monitoring the prevalence of methamphetamine-related presentations at psychiatric hospitals in Cape Town, South Africa. *African journal of psychiatry*. *16*(1):45-49.

Pok, P.R.P., Mauras, M., De Saint Léger, M.N., Kuhlmann, E., Charpenel-Durat, C., Navarette, C., Duval, M.L. & De Meo, P. 2010. Blood concentrations of clobazam and norclobazam in a lethal case involving clobazam, meprobamate and clorazepate. *Legal medicine. 12*(6):300-304.

Pompili, M., Serafini, G., Innamorati, M., Dominici, G., Ferracuti, S., Kotzalidis, G.D., Serra, G., Girardi, P., Janiri, L., Tatarelli, R. & Sher, L. 2010. Suicidal behavior and alcohol abuse. *International journal of environmental research and public health*. *7*(4):1392-1431.

Rassool, G.H. & Winnington, J. 2006. Psychoactive Substances and their Effects. *Dual Diagnosis Nursing.* 34-44.

Rataemane, S. & Rataemane, L. 2006. Alcohol in South Africa. *International Journal of Drug Policy*. *4*(17):373-375.

Ray, W.A., Meredith, S., Thapa, P.B., Hall, K. & Murray, K.T. 2004. Cyclic antidepressants and the risk of sudden cardiac death. *Clinical Pharmacology & Therapeutics*. 75(3):234-241.

Reid, K.M., Martin, L.J. & Heathfield, L.J. 2020. Bodies without names: A retrospective review of unidentified decedents at Salt River Mortuary, Cape Town, South Africa, 2010 - 2017. *South African medical journal.* 110(3):223–228. DOI: 10.7196/SAMJ.2020.v110i3.14192.

Republic of South Africa. *Health Professions Act (Act 56 of 1974)* (and its amendments). Pretoria: Government Printer; 1974. Available: Medical, Dental and Supplementary Health Service Professions Act 56 of 1974 | South African Government (www.gov.za) [2023, March 01].

Republic of South Africa. *National Health Act (Act No. 61 of 2003).* Pretoria: Government Printer; 2003. Available: https://www.gov.za/documents/national-health-act [2023, March 02].

Republic of South Africa. *National Road Traffic Act (Act 93 of 1996).* Pretoria: Government Printer. 1996. Available: National Road Traffic Act 93 of 1996 | South African Government (www.gov.za) [2023, March 01].

Republic of South Africa. *The Inquests Act (Act 58 of 1959) (and its amendments).* Pretoria: Government Printer. 1959. Available: Inquests Act 58 of 1959 | South African Government (www.gov.za) [2023, March 02].

Rivara, F.P., Mueller, B.A., Somes, G., Mendoza, C.T., Rushforth, N.B. & Kellermann, A.L. 1997. Alcohol and illicit drug abuse and the risk of violent death in the home. *Jama.* 278(7):569–575.

Robertson, M.D. & Drummer, O.H. 1995. Postmortem drug metabolism by bacteria. *Journal of Forensic Sciences*. *40*(3):382-386.

Rock, N.L. & Moore, R.J. 1976. Methaqualone (Mandrax) abuse, urine testing, and identification: Clinical correlation between a new mass urinalysis test and a military drug abuse program. *International Journal of the Addictions*. *11*(2):237-244.

Rossiter, D. 2017. South African Medicines Formulary. 11th ed. D. Rossiter & M. Blockman, Eds. Cape Town, SA: Health and Medical Publishing Group.

San Nicolas, A.C. & Lemos, N.P. 2015. Toxicology findings in cases of hanging in the City and County of San Francisco over the 3-year period from 2011 to 2013. *Forensic Science International*. 255:146-155.

Schuckit, M.A. 2011. Ethanol and methanol. *Goodman and Gilman's the pharmacological basis of therapeutics. 12th ed. New York: McGraw-Hill*. 629-47.

Schulz, M., Schmoldt, A., Andresen-Streichert, H. & Iwersen-Bergmann, S. 2020. Revisited: Therapeutic and toxic blood concentrations of more than 1100 drugs and other xenobiotics. *Critical Care. 24:*1-4.

Schütz, H., Paine, A., Erdmann, F., Weiler, G. & Verhoff, M.A. 2006. Immunoassays for drug screening in urine: Chances, challenges, and pitfalls. *Forensic Science Medicine and Pathology*. *2*:75-83.

Sheehan, C.M., Rogers, R.G., Williams IV, G.W. & Boardman, J.D. 2013. Gender differences in the presence of drugs in violent deaths. *Addiction*. *108*(3):547-555.

Sinha, A., Lewis, O.D., Kumar, R., Yeruva, S.L.H. & Curry, B.H. 2016. Amphetamine abuse related acute myocardial infarction. *Case Reports in Cardiology*. *2016*(1):7967851.

Skopp, G. 2004. Preanalytic aspects in postmortem toxicology. *Forensic Science International.* 142(2-3):75-100.

Skopp, G. 2010. Postmortem toxicology. *Forensic Science, Medicine, and Pathology*. 6(4):314–325.

South African Community Epidemiology Network on Drug Use (SACENDU). 2022. *Annual report.* Available: SACENDUBriefPhase50.pdf (host-h.net) [2023, December 04].

South African Police Service (SAPS). 2021/22. Annual report. Available: https://www.gov.za/sites/default/files/gcis_document/202211/saps-2021-22.pdf [2023, December 04].

Statistics South Africa. 2012. *Cape Town census and population statistics*. In.: City of Cape Town Information and Knowledge Management Department; City of Cape Town. Available: capetown.gov.za/Family and home/education-and-research-materials/data-statistics-and-research/cape-town-census/ [2023, December, 14].

Stewart, M.J., Stewart, M., Moar, J.J., Mwesigwa, J. & Kokot, M. 2000. Forensic toxicology in urban South Africa. *Journal of Toxicology: Clinical Toxicology*. *38*(4):415-419.

Tascon, I., Côté, C., Garneau, B., Desharnais, B., Gosselin, V. & Mireault, P. 2023. Postmortem redistribution of cannabinoids: Statistical analysis of a novel dataset and meta-analysis. *Forensic Science International*. *353*:111873.

Thennakoon, J.C. 2025. Unveiling the Impact of Illicit Drug Addiction on Immune Suppression: A Narrative Review. *Eurasian Journal of Science and Technology*. *5*(1).

Thomson, K. 2013. *Overview of the drug abuse problem in South Africa*. Available: http://www.harmonygroup. co.za/drugs/overview-of-the-drug-abuse-problem-in-south-africa/ [2023, March 01].

Tominaga, M., Michiue, T., Inamori-Kawamoto, O., Hishmat, A.M., Oritani, S., Takama, M., Ishikawa, T. & Maeda, H. 2015. Efficacy of drug screening in forensic autopsy: Retrospective investigation of

routine toxicological findings. *Legal Medicine.* 17(3):172–176. DOI: 10.1016/j.legalmed.2015.01.001.

United Nations Office on Drugs and Crime (UNODC). 2021. *World Drug Report 2021.* New York: United Nations, 2021. Available: http://www.unodc.org/documents/data-and-analysis/WDR2021/World_Drug_Report_2021_ebook.pdf [2023, March 02].

United Nations Office on Drugs and Crime (UNODC). 2022. *World Drug Report 2022.* New York: United Nations, 2022. Available: http://www.unodc.org/documents/wdr2022/World_Drug_Report_2022_web.pdf [2023, February 28].

Vasudevan, V. 2019. Six-Week-Old Girl Dies of Methamphetamine Toxicity After being Breastfed By Drug addict Mother. Available: Six-week-old girl dies of methamphetamine toxicity after being breastfed by drug addict mother | MEAWW [2023, Jan 6].

Vitale, S. & van de Mheen, D. 2006. Illicit Drug Use and Injuries: A Review of Emergency Room Studies. *Drug Alcohol Depend.* 82 (1):1−9.

Volkow, N.D. 2014. America's addiction to opioids: Heroin and prescription drug abuse. *Senate Caucus on International Narcotics Control. 14:*1-16.

Vos, T., Lim, S.S., Abbafati, C., Abbas, K.M., Abbasi, M., Abbasifard, M., Abbasi-Kangevari, M., Abbastabar, H., Abd-Allah, F., Abdelalim, A. & Abdollahi, M. 2020. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet. 396*(10258):1204-1222.

Watt, M.H., Myers, B., Towe, S.L. & Meade, C.S. 2015. The mental health experiences and needs of methamphetamine users in Cape Town: A mixed-methods study. *South African Medical Journal. 105*(8):685-688.

Wechsberg, W.M., Myers, B., Kline, T.L., Carney, T., Browne, F.A. & Novak, S.P. 2012. The relationship of alcohol and other drug use typologies to sex risk behaviors among vulnerable women in Cape Town, South Africa. *Journal of AIDS & clinical research*. (15).

Western Cape Government. 2016. Western Cape *injury profile* (2010-2016). Available: https://www.westerncape.gov.za/assets/departments/health/mortality_profile_2016.pdf [2023, December 02].

Western Cape Government. 2022. Western Cape *Forensic Pathology Service*. Available: ttps://www.westerncape.gov.za/general-publication/our-facilities [2023, November 01].

Westover, A.N., Nakonezny, P.A. & Haley, R.W. 2008. Acute myocardial infarction in young adults who abuse amphetamines. *Drug and Alcohol Dependence*. *96*(1-2):49-56.

Wetli, C.V. 2007. Pathology of drug abuse. In: Karch SB, ed. *Drug Abuse Handbook*. 2nd ed. Boca Raton, Fla: CRC Press. 72-145.

Wolf, B.C., Lavezzi, W.A., Sullivan, L.M., Middleberg, R.A., Flannagan, L.M. 2005. Alprazolam-related deaths in Palm Beach County. *The American Journal of Forensic Medicine and Pathology*. 26:24–27.

World Health Organization (WHO). 2015. Global status report on road safety 2015, Geneva, *Switzerland: World Health Organization.* Available http://www.who.int/violence_injury_prevention/road_safety_status/2015/en/ [2023, April 06].

Wu, A.H., Wong, S.S., Johnson, K.G., Callies, J., Shu, D.X., Dunn, W.E. & Wong, S.H. 1993. Evaluation of the triage system for emergency drugs-of-abuse testing in urine. *Journal of Analytical Toxicology*. *17*(4):241-245.

Yu, F., Lian, X., Zhang, H., Xie, M. & Zhang, L. 2015. Quantitative Analysis of Heroin and its Metabolites in vivo. *Scandinavian Journal of Laboratory Animal Science*. *41*:1-6.

Appendix A: Analytes and Internal Standards in the Targeted LC-MS/MS Panel

	Analyte	Blood Limit of Quantitation (LOQ) (mg/L)	Urine Limit of Detection (LOD)
1	6-AM	0.010	0.010
2	Acetaminophen	0.100	N/A
3	Alprazolam	0.005	0.005
4	Amitriptyline	0.020	0.050
5	Amphetamine	0.020	0.050
6	Benzoylecgonine	0.020	0.050
7	Buprenorphine	0.004	0.010
8	CBD	N/A	0.020
9	Clobazam	0.010	0.010
10	Cocaethylene	0.020	0.050
11	Cocaine	0.020	0.050
12	Codeine	0.010	0.020
13	Diazepam	0.020	0.010
14	Diphenhydramine	0.040	0.050
15	Fentanyl	0.001	0.001
16	Hydrocodone	0.010	0.020
17	Hydromorphone	0.010	0.020
18	Ketamine	0.020	0.050
19	MDA	0.010	0.025
20	MDMA	0.010	0.025
21	Methadone	0.020	0.050
22	Methamphetamine	0.020	0.025
23	Methaqualone	0.050	0.050
24	Methcathinone	0.020	0.020
25	Morphine	0.010	0.020
26	O-DSMT	0.010	0.100
27	Oxycodone	0.010	0.050
28	Oxymorphone	0.010	0.020
29	THC	N/A	0.020
30	THC-COOH	0.010	0.020
31	Tramadol	0.020	0.050

Appendix B: Internal Standards Used in the Method

#	Internal standard (Blood)	Internal Standards (Urine)
1	6-Acetylmorphine-d3	Diazepam-d5
2	Acetaminophen-d4	Doxepin-d3
3	Amphetamine-d6	
4	Benzoylecgonine-d3	
5	Buprenorphine-d4	
6	Cocaethylene-d3	
7	Cocaine-d3	
8	Codeine-d3	
9	Diazepam-d5	
10	Fentanyl-d5	
11	Hydrocodone-d3	
12	Hydromorphone-d3	
13	Ketamine-d4	
14	MDA-d5	
15	MDMA-d5	
16	Methadone-d3	
17	Methamphetamine-d5	
18	Morphine-d3	
19	O-desmethyl-cis-tramadol-d6	
20	Oxycodone-d3	
21	Oxymorphone-d3	
22	Tramadol-13C-d3	

Appendix C: Ethics Approval Letter

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

Room 45 E-52-E-Floor- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/home/human-research-ethics

02 May 2023

HREC REF: 266/2023

Ms B Davies
Division of Forensic Medicine & Toxicology
Room 5.07 Falmouth Building-FHS
Email: Bronwen.davies@uct.ac.za
Student: Mnrmur002@myuct.ac.za

Dear Ms Davies

PROJECT TITLE: INVESTIGATING THE ROLE OF COMMONLY MISUSED DRUGS IN UNNATURAL DEATHS IN CAPE TOWN IN 2022-
(MPHIL CANDIDATE-MR MURENDWA MUNARINI)

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, subject to the following:

1. Updating the letter to the HREC to Helsinki Dec of 2013 from 1964 and adding to the application form R006/2021.

Approval is granted for one year until the 30 May 2024.

Please submit a progress form, using the standardised Annual Report Form (FHS016) 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)

The HREC acknowledge that the student: Mr Murendwa Munarini will also be involved in this study.

Please quote HREC REF 266/2023 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

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

HREC/ref 266.2023

Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938 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 2020), 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.

HREC/ref 266.2023