A Post-Mortem Toxicological Investigation: Understanding the Role of Drugs of Abuse in Violent Fatalities in Cape Town, South Africa

By

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Declaration

I, ***Marie Belle Kathrina Mendoza Auckloo***, hereby declare that the work on which this dissertation 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.

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Abstract

Violence and resulting injuries are critical health burdens worldwide, accounting for the death of millions of individuals annually. The literature reports an association between drug use and violence, providing data indicating that the use of psychoactive substances increases the risk of morbidity and mortality due to violent acts. South Africa has a long history of violence, with one of the highest rates of recorded violence- and injury-related deaths in the world. This is complicated by an increase in illicit substance use and abuse, particularly in the Cape Town Metropole, located within the Western Cape Province.

The use of toxicological findings from victims of violent death (homicides, suicides, and accidents) to examine community-specific drug-related violence is slowly increasing in different parts of the world. In South Africa, however, monitoring drug trends in violent fatalities using toxicological analysis is uncommon, and hence drug toxicology of violent-related fatalities is limited.

Divided into three contextual sections, this research study focuses on the post-mortem toxicology of violent deaths in a South African setting. The first section provides a general idea of the research problem and an initial development of the investigation process. The second section provides a theoretical basis for performing routine toxicological analyses in deaths due to violence, reports important research work conducted in the field worldwide, and emphasizes the need to monitor toxicological data derived from violent fatalities in Cape Town, South Africa. The last section, in the form of a manuscript, presents the overall research study including the methodology, outcomes, and concluding findings in a concise and illustrative manner.

The primary aim of this pilot study was to investigate the prevalence and characteristics of illicit substances in violent fatalities (homicides, suicides, and accidents) of the Salt River mortuary in Cape Town, South Africa. The objectives were to conduct a comprehensive drug toxicology analysis to generate qualitative and comparative data from the aforementioned cases. In addition, this study investigated the dynamics between psychoactive substance use and violent deaths in terms of toxicological trends, and the demographics and circumstances of death of the victim. Lastly, the author discusses potential qualitative associations between illicit substances and violence-related deaths in a South African setting, and provide suggestions for future toxicological analyses in these fatalities.

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List of Abbreviations

The following table lists abbreviations and acronyms used throughout the dissertation.

Abbreviation or Symbol	Meaning
ACC	Accidental or Unintentional death group (in this study)
ATS	Amphetamine-type Stimulants
CN	Cyanide
CNS	Central Nervous System
CO	Carbon Monoxide
DUI	Driving under the influence
FCL	Forensic Chemistry Laboratory
FPO	Forensic Pathology Officer
FPS	Forensic Pathology Services
GABA	Gamma-Aminobutyric Acid (or γ-Aminobutyric Acid)
HOM	Homicide group (in this study)
HPLC	High Performance Liquid Chromatography
HREC	Human Research Ethics Committee
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
MDMA	3,4-methylenedioxy-methamphetamine
M3G	Morphine 3-β-D-glucuronide
MPA/B	Mobile Phase A/B
NaF	Sodium Fluoride
NIMSS	National Injury Mortality Surveillance System
NPS	Novel (or new) Psychoactive Substances
OTC/PRE	Over-the-counter and Prescription Drugs
PCP	Phencyclidine
SA	South Africa
SACENDU	South African Community Epidemiology Network on Drug Use
SAPS	South African Police Services
SRM	Salt River Mortuary
SUI	Suicide group (in this study)
THC	Tetrahydrocannabinol
THC-COOH	11-nor-9-carboxy-Δ9-tetrahydrocannnabinol
UND	Undetermined death group (in this study)
UNODC	United Nations Office on Drugs and Crime
UCT	University of Cape Town
WHO	World Health Organization
11-OH-THC	11-Hydroxy-Δ9-tetrahydrocannabinol

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Part A

Research Proposal

Introduction

Violence and resulting injuries present critical worldwide health and public safety issues which greatly affect developing countries, such as South Africa. In addition to high rates of violence-related fatalities, the country is experiencing an increase in substance – a drug, medicine, or other chemical agent which has physiological effect when introduced into the body – use and abuse, particularly in the Western Cape Province. A relationship between substance use and violent injury deaths has been reported in the literature. However, toxicological data on drugs of abuse in victims of violence-related injury deaths is limited.

This research project aims to identify and describe the possible relationship between substance use and deaths due to homicide, non-substance toxicity-related suicides, and accidental deaths, based on descriptive qualitative prevalence, in a South African setting by conducting a comprehensive toxicological analysis of these cases from the Salt River mortuary in the Cape Metropole. Over a set period of time, biological specimens important for cause of death interpretation will be collected from decedents in the aforementioned cases as part of the routine toxicology investigation with the help of forensic pathologists. A targeted drugs of abuse screening approach using liquid chromatography coupled to tandem mass spectrometry (AB SCIEX API 3200 Q-TRAP® LC-MS/MS) will be used to perform a toxicological screening of the samples collected. The data obtained will be analysed initially using the AB SCIEX MasterView™ software and subsequently by statistical inferences.

The main objectives of the study are to distinguish, and make comparisons on, the prevalence of drugs of abuse (substances commonly abused, which may lead to physical or psychological dependence) in the cases of interest, and to discuss the dynamics between drugs of abuse and violent fatalities. This study will contribute to the much needed understanding of violent fatality patterns and related toxicology, and will provide essential data for health, drug and crime interventions, in the attempt to reduce these burdens in South Africa.

Background

2.1 Violence and Injury

Violence and resulting injuries are major public health issues worldwide. In this study, violence refers to the use of physical force against another person or oneself to cause injury and even death [1]. Violence related to assault, self-inflicted injury or acts of war often result in serious injuries and death of more than 5 million people worldwide annually, accounting for approximately 10% of global mortality [2]. Violence and injury-related fatalities may be a result of collective, interpersonal and self-directed violence in the form of transport accidents, homicides, suicides, unintentional or accidental deaths, and fatal injuries of undetermined intent. Deaths due to unintentional injury result from a variety of causes such as falls, drowning, substance overdoses, asphyxiation, choking, animal bites or stings, fires or burns, firearm accidents, and accidental blunt or sharp force trauma. An undetermined violent death refers to a traumatic death in which the forensic pathologist cannot determine with certainty the manner of the death.

The manner of death refers to the circumstances surrounding the death of an individual and this can be classified as natural, homicide, suicide, accident, or undetermined [3]. The cause of death refers to the disease, condition or injury that leads, directly or indirectly, immediately or subsequently, to the death of the person [3]. The four leading manners of injury and violence-related fatalities worldwide are transportation incidents, suicides, homicides, and falls as shown in Figure 1 [4,5]. More than 90% of these fatalities occur in low- and middle-income regions, such as in African countries [5,6]. South Africa (SA) is confronted with a remarkable burden of morbidity and mortality as a consequence of violence, which constitutes one of the four prominent health burdens in the country [7]. Violence-related injury was reported to be the second leading cause of death in SA in 2000 with an overall mortality rate of 157.8 per 100,000 individuals, which is almost double that of the global average (86.9 per 100 000 individuals) [8,9]. Of a total of 31,777 unnatural deaths recorded in 2008, murder (homicide) was the leading manner of death (*n*=9831, 31.5%), as shown in Figure 2 [10]. Unintentional or accidental deaths, suicides and undetermined deaths accounted for 17.5% (n=5444), 10% (n=3125), and 11.6% (n=3624), respectively [10].

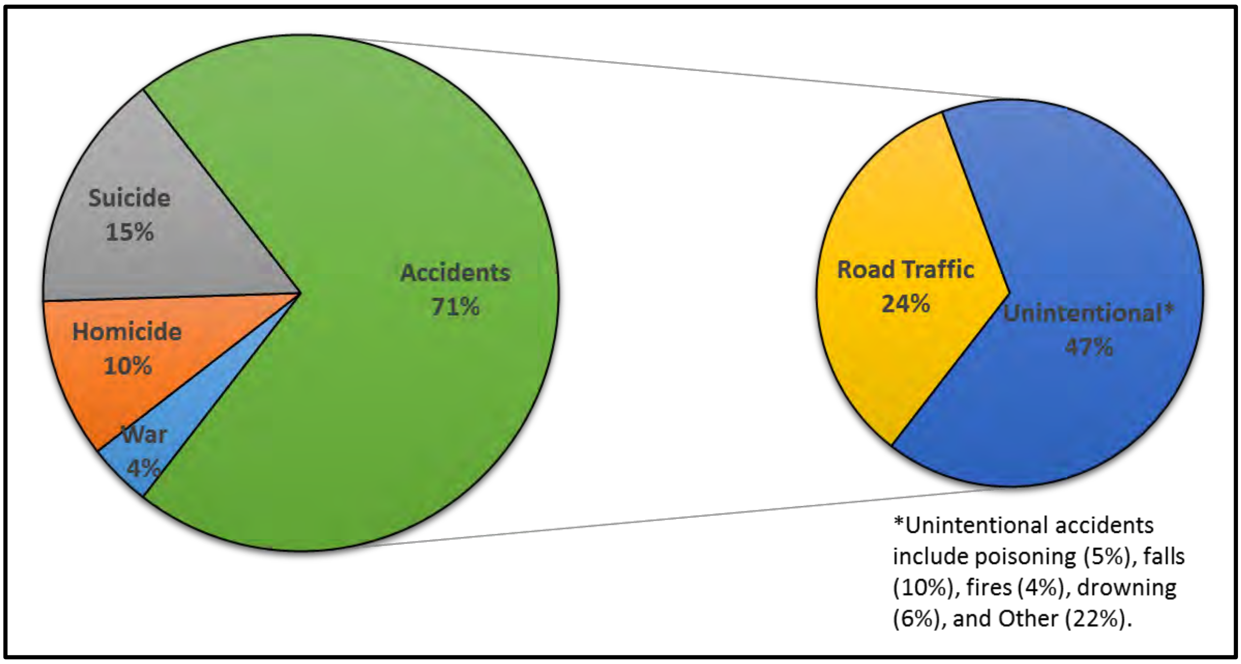

Fig.1 Proportion of world injury mortality by cause of death in 2008 [4,5].

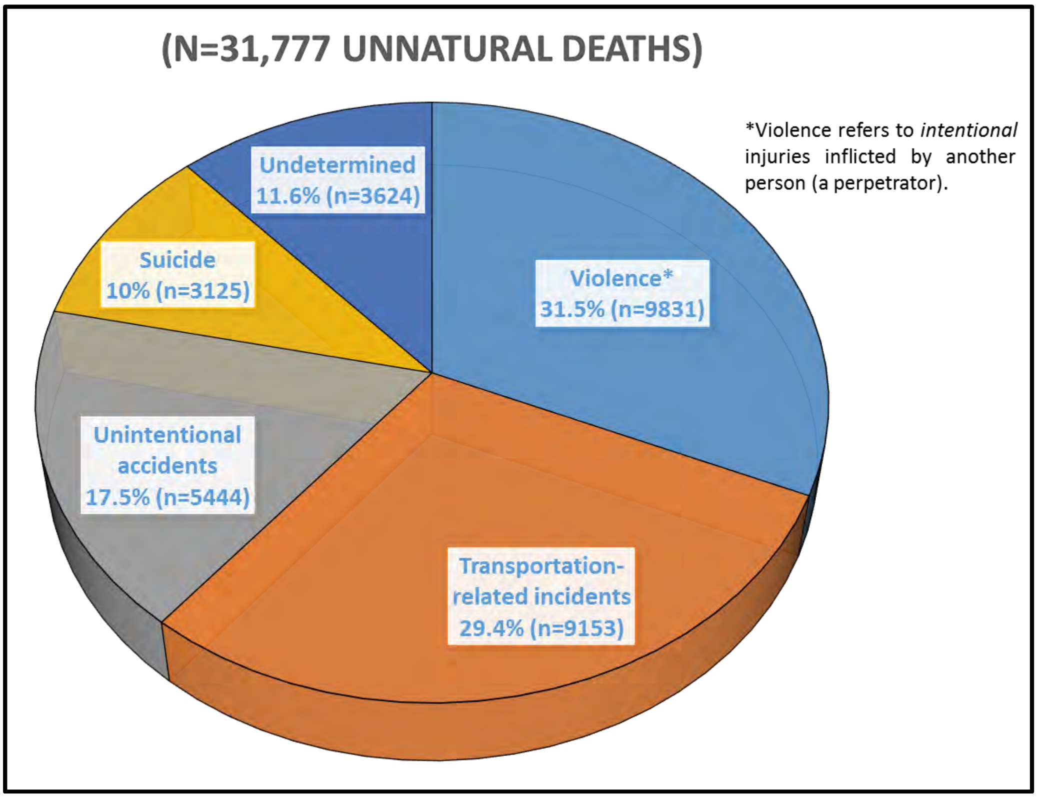

Fig.2 Overall manner of death due to unnatural causes reported in South Africa in 2008 [10].

In the Western Cape Province of SA, violence accounted for about 13% of fatalities in 2000 [7]. In 2007, the Cape Town Metropole had both the highest injury mortality and violence rates compared to other South African metros [11]. Homicide accounted for 46.9% of unnatural deaths with unintentional or accidental injuries (12.6%) and suicide (7.8%) also deemed to play a large role in unnatural deaths (Fig. 3) [11]. In 2010, 64.5 % of injury-related fatalities reported in the Western Cape occurred in Cape Town, and the highest proportion of unnatural deaths were homicides (41%) [12].

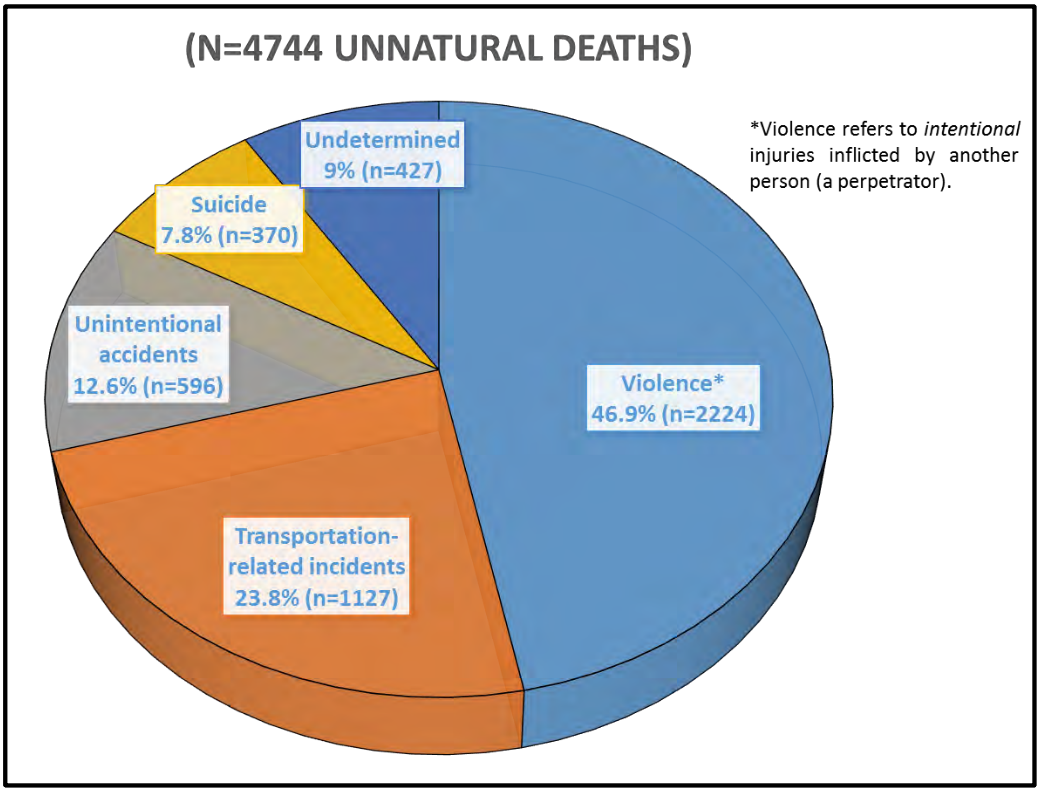

Fig.3 Overall manner of death due to unnatural causes reported in Cape Town in 2007 [11].

2.2 *Drugs of Abuse*

In 2012, approximately 243 million people, aged 15-64 years, were reported having used an illicit substance that year [13]. The proportion of known drug users is reported to be roughly 0.6% of the world's adult population, which numbers to around 27 million individuals, or 1 in every 200 people [13]. Cannabis is reportedly the most widely used illicit substance worldwide, followed by amphetamine-type stimulants (ATS) (including methamphetamine, amphetamine, and methcathinone), opioids (such as opiates, heroin, and prescription opioids), and cocaine [14]. There remains a dearth of reliable and comprehensive data on substance abuse in Africa, however limited information obtained from research and treatment centres reveals that in SA, alcohol is the most abused substance, while cannabis is the most widely used illicit drug, particularly amongst young adults [13,15,16]. In the Western Cape, the most widely used illicit drug is methamphetamine, followed by marijuana and heroin [15,17]. Research indicates an increase in the rate of drug-related crime in Cape Town over the period 2003/2004 to 2012/2013, from 306 to 1495 reported crimes, which represents an increase of 479% in actual reported crimes over the 9-year period and an average of 24% per annum [17]. Cape Town is currently battling a widely publicized emerging methamphetamine (locally known as 'tik') problem, especially amongst the young population, of which 40% of treatment centre patients are younger than 25 years old [15,18]. 'Tik' remains the substance of choice, accounting for 28% of reported patient admissions, in Cape Town [15]. This is followed by cannabis ('dagga'), alcohol, heroin, methcathinone ('cat'), methaqualone ('mandrax'), and cocaine ('crack') use.

2.3 Substance Use and Violent Deaths

Studies suggest an association between psychoactive substance – a substance, used for medical or recreational purposes, that primarily acts on the central nervous system to alter mental processes including perception, consciousness, behaviour, mood, and thoughts – abuse, including alcohol and illicit drugs, and increased levels of violence [19,20]. Substance dependence and tolerance (the physiological adaptation to the drug and its effects at a given dosage) increases the risk of violence, which is suggested to contribute to elevated homicide mortality rates among substance users [21]. Licit and illicit substance-dependent populations have shown to exhibit higher all-cause death rates compared to non-drug-dependent populations [22]. For instance, alcohol dependence is associated with a 2-fold increase in all-cause mortality [22]. Likewise, scheduled drugs, such as benzodiazepines and opioids have also been shown to be associated with elevated violence-related death rates [22]. While disease and drug overdose are the *primary* causes of death associated with substance dependence, drug use may result in increased violence, crime and mortality associated with *traumatic* primary causes of death [20,23,24]. Altogether, the literature recognizes that a relationship exists between substance abuse and increased occurrence of violent deaths.

2.3.1 <u>Toxicology of Homicides</u>

In 2012, almost half a million reported deaths worldwide, of which 31% occurred in Africa, were reported as homicides [25]. The global average homicide rate is currently 6.2 per 100,000 individuals, and Southern Africa's reported rates are over four times than that (e.g.

30 per 100,000 residents in 2011), making it one of the world's sub-regions with the highest recorded homicide rates [25]. Studies and available data indicate that the use of licit and illicit drug may be correlated with high levels of violence and homicide [25]. So far, studies investigating the toxicology in homicidal deaths have found that alcohol and illicit drugs are present in over 50% of the reported cases [26–30]. A study in New York, 1990-1998, revealed that 30% of the homicide victims appear positive for alcohol, 28% for cocaine, 19% for cannabis, and 11% for opiates [30]. Similarly, a toxicological investigation of homicide victims in Australia 1996-2005 found that psychoactive drugs were detected in 62.6% of cases, and other illicit substances in 32.8% [21]. In a Swedish setting, toxicology findings of homicide deaths from 2007 to 2009 indicate that the majority of the cases (57% of victims and 62.5% of offenders) tested positive for drugs of abuse, such as alcohol and benzodiazepines [31]. Toxicological data have clearly shown to provide useful information for investigating drug use patterns in homicidal deaths and the associated risk reported between drug abuse and victim/offender status in violent crimes.

2.3.2 Toxicology of Suicides

Suicide is one of the major global manners of death with a mortality rate of approximately one million deaths per year [12]. Substance-dependent populations are known to be at a higher risk of completed suicide compared to the general population [32–34]. However, few studies around the world have been examining substance levels in completed suicide victims [33,35–39]. Among the suicide cases, over 50% of cases were positive for drugs of abuse, including alcohol and illicit drugs, however pharmaceuticals (benzodiazepines and antidepressants) appear to be detected more frequently in suicides compared to homicide cases [33,35,37–39]. In cases of substance overdose/poisoning-related suicides, the relationship between violent death and drug use is easily made clear through toxicological analyses and interpretations. However, the link between non-substance related suicide cases and post-mortem toxicology is more complex and still unclear [33,40–42]. In 2009, Darke *et al.* investigated patterns of substance use in suicide deaths by means other than substance overdose, and reported the presence of substances in 67.2% of non-overdose cases [33]. Alcohol was found in 40.6% of cases and illicit substances in 20.1% [33]. Although the information from the literature indicates that toxicology analyses is crucial in the medico-legal investigation of suicidal deaths, there remains a dearth of information and emphasis is placed on the necessity of further studies to better understand the dynamics of drug use and suicide.

2.3.3 Toxicology of Unintentional and Undetermined Deaths

Deaths due to unintentional fatal injuries are those in which the manner of death is ruled to be an accident. Over 3.9 million deaths worldwide in 2004 was a consequence of unintentional injuries, with 90% of those occurring in low- and middle-income countries, such as South Africa, Ghana, Colombia, Uruguay, Egypt, Afghanistan, Croatia, China, among others [43]. Excluding transportation (land, air, and water) incidents, the global leading causes of accidental or unintentional injury deaths are falls, drowning, and substance overdose [43]. In the USA in 2008, about 77% of the drug overdose deaths were unintentional, 13% were suicides, and 9% were of undetermined intent [44]. Undetermined deaths refers to fatalities for which the manner of death cannot be legally determined with, therefore it is unknown if it is an accident or suicide case [45]. Several studies have shown that many suicides are misclassified as undetermined, and hence the reported suicide rates may underestimate the true rate [45,46].

2.4 Fatalities in the Cape Metropole

The definitions of cause and manner of death are often used interchangeably by clinicians, professionals in the forensic field, epidemiologists, nosologists, and laymen, however but they are not synonymous. In SA, the Inquest Act (Act 58 of 1959) provides for the holding of inquests in cases of deaths occurring from other than natural causes. Under this Act and in accordance with the regulations of the National Health Act (Act 61 of 2003), a person who has allegedly died from unnatural causes shall be examined by the medical practitioner who performs a post-mortem examination of the body to assist the court in medico-legal investigations. The forensic pathologists of the Forensic Pathology Services (FPS) are responsible for determining the cause and manner of death in theses non-natural deaths in SA. The Salt River mortuary (SRM) situated in Salt River in Cape Town, is a medico-legal mortuary that serves the West Metropole of Cape Town and handles on average a case load of 3000 autopsies per year [47]. This study focuses on cases of suspected non-substance related suicide, homicide, and unintentional or undetermined violent deaths from the SRM. All unnatural death cases undergo a standardized forensic autopsy performed by the forensic pathologists, in which biological specimens for toxicology and histology analyses may be collected if deemed necessary by the pathologist. The cause and manner of death is subsequently determined by analysing all evidence pertaining to the circumstances of death and the decedent's history, the autopsy findings, and the results of requested additional analyses.

Study Rationale

SA has a long history of violence, with one of the highest rates of recorded violence-related deaths, including homicides and suicides, in the world [48,49]. This is complicated by an increase in illicit substance use and abuse, such as the high rate of methamphetamine abuse in the Cape Town [16,18]. The availability of recent and reliable data on licit and illicit drug use and abuse in developing countries is still a challenge for forensic and epidemiology researchers. To date, relatively few studies have investigated post-mortem drug concentrations in victims of homicide, completed suicide, accidental and undetermined intent [21,29–31,34,39]. And more importantly, few have performed statistical comparative analyses of toxicological data between these case types [22]. In SA, toxicological studies on homicides, non-overdose suicide, and accidental and undetermined injury intent victims are non-existent. Consequently, the association between drugs of abuse and these types of violence-related deaths in a South African setting is unknown. Due to high fatality rates in the Western Cape, of which violence largely contributes, toxicological investigation of drug use patterns in these cases is imperative, particularly given the well-documented link between psychoactive substance use and increased violence [7,11,12,19,50–53].

In SA, toxicological examinations of post-mortem tissue and blood samples are conducted by the National Forensic Chemistry Laboratory (FCL) in cases of unnatural or unexpected deaths. Blood and other biological samples are usually collected in homicide, non-overdose suicide, and unintentional and undetermined deaths at autopsy. However, at present, while toxicological testing of biological samples in all unnatural deaths is suggested within the national protocol, it is not routinely performed due to the limited resources, large number of unnatural fatalities, case backlogs, and other challenges currently experienced by the laboratory, mortuary and related agencies. Again, this is not routine due to the above-mentioned limitations. This study focuses on the toxicology in suspected homicide, non-substance related suicide, and unintentional/accidental and undetermined violent fatalities in a South African setting in an attempt to provide a quantitative description of drugs of abuse in these particular deaths over a set period of time. This research will form the basis for future large-scale research in post-mortem forensic toxicology in SA. The systematic and cumulative increase in knowledge regarding the dynamics of substance use and violent deaths would generate more toxicological evidence to the benefit of forensic researchers and practitioners in the fields of forensic pathology and toxicology.

Aim and Objectives

The ultimate goal of this investigation is to generate qualitative and comparative toxicological data which will provide potentially valuable insight for future research in the field. Therefore, the purpose of this study is to conduct a comprehensive toxicological analysis of violence-related homicides, non-overdose completed suicides, unintentional accidents, and undetermined fatalities, over a set period of time, in Cape Town, SA. To this end, the objectives are to:

1. Analyse the prevalence of drugs of abuse in the aforementioned cases by screening for drugs of abuse and conducting necessary confirmatory liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses on collected post-mortem biological samples, primarily blood and urine specimens.

2. Perform a statistical comparative and quantitative descriptive analysis of toxicology these fatalities.

3. Analyse and describe toxicological patterns, demographic characteristics, and circumstances of death in these violent fatalities.

4. Critically discuss potential qualitative associations between substance use and violence-related fatalities providing suggestions for future research.

5. Provide suggestions based on the information obtained for drug abuse policy interventions as well as procedure-related alterations of current toxicology analysis strategies in these violent fatalities.

Proposed Methodology

5.1 Research Design

5.1.1 <u>Sample Sources and Population</u>

The study population of interest in this investigation includes all Cape Metropole fatalities that are sent to the SRM during the research period, in which the *suspected* manner of death is homicide, non-substance toxicity-related suicide, accidental or undetermined. The *suspected* manner of death refers to the apparent intention prior to the fatal injury that resulted in the death of an individual. This is determined by the police, forensic pathology officers who attend the death scene, and the forensic pathologists following a post-mortem examination of the body. The *final* manner of death is only determined after laboratory analyses and Inquest court proceedings, which can take several years to complete [11,10].

5.1.2 <u>Criteria and Parameters</u>

Due to the novel (especially in SA) nature of this study, the sample size of the population of interest is estimated to be within the range N=300-500 based on review of data obtained from the SRM databases in 2013-2014 for the period June to October. The inclusion criteria for this study population are consecutive cases of suspected homicide (HOM group), non-overdose completed suicide (SUI group), unintentional/accidental death (ACC group), and undetermined death (UND group) from the SRM from June to October 2015. Exclusion criteria are deaths due to substance-related toxicity which may include overdose of therapeutic and/or recreational drugs, poisonings due to ingestion of pesticides, acids and other chemicals, as well as gassing by means of carbon monoxide and other gases or volatile inhalants. This is so as to not bias the toxicology results through inclusion of persons for whom drugs might have been the *primary* cause of death. Road traffic accidents will also be excluded from the study as they lie in a separate category of violent deaths, and will be observed in different studies in future.

5.2 Specimen Sampling

Samples will be collected during the routine autopsies performed by the forensic pathologists at SRM. With the assistance of the forensic pathology officers (FPO's), the specimens listed in Table 1 will be collected in the appropriate containers using standard national and international guidelines in terms of quantities and sampling techniques [54]. Sealed in evidence bags, the specimens collected will be transported and stored in the Pharmacology Laboratory in the University of Cape Town (UCT), Division of Clinical Pharmacology at the Groote Schuur Hospital.

Table 1 Specimen collection quantities, containers and storage conditions.

Specimen	Quantity	Container	Storage Condition
1. Femoral Blood	20 mL	Grey top tube containing Sodium Fluoride (NaF)	4°C
2. Heart Blood	20 mL	NaF Grey top tube	4°C
3. Urine	As much as possible (50 mL)	Falcon tube (Blue top)	4°C
4. Vitreous Humor	All	Hospital tube (red top)	4°C
5. Hair	Pencil-sized scalp hair	Collected in paper and secured using druggist fold	Room temperature
6. Bile	All	Falcon tube (Blue top)	-20°C
7. Liver	50 g	Falcon tube (Blue top)	-20°C
8. Gastric Content	All (or 50g homogenised)	Falcon tube (Blue top)	-20°C

5.3 Systematic Qualitative Toxicology Analysis

5.3.1 Sample Preparation for Qualitative Analyses

Blood sample preparation involves acetonitrile protein precipitation of low sample volumes combined with aqueous dilution [55]. Urine samples will be prepared using a simple "dilute and shoot" method which is coupled with a highly sensitive LC-MS/MS system [56]. Deuterium-labelled internal standards (Diazepam-D_5 and Doxepin-D3) at known concentrations will be added to urine (diluted) before analysis. Full scan product ion spectra will be obtained in the data-dependent mode using a linear ion trap (API 3200 Q-trap).

5.3.2 Instrument

A Shimadzu Prominence High Performance Liquid Chromatography system coupled to an AB SCIEX API 3200 Q-TRAP Mass Spectrometer at the UCT Pharmacology Laboratory will be used for qualitative drugs of abuse screening of the biological samples collected. The data obtained will be processed and analysed by the researcher using the AB SCIEX MasterView™ software.

5.4 Data Analysis and Interpretation

5.4.1 Data Management

All the information obtained from post-mortem toxicology forms, autopsy reports, and toxicological analyses will be extracted for each case and recorded and stored securely in a controlled access database.

5.4.2 Statistical Analysis

Statistical analysis will be carried out on data obtained using STATA software. Descriptive frequencies and means for all variables of interest will be presented. For discrete/categorical variables, the data will be described and summarised using frequency counts and percentages. For continuous variables, data will be described and summarised using means, standard deviations, medians, quartiles and ranges as appropriate.

Confidence intervals (95%) will be calculated for prevalence proportions. For comparisons between groups (e.g. male vs. female) the appropriate statistical techniques will be performed: paired or independent t-tests or Wilcoxon Rank Sum test for comparison of means and medians/distributions respectively. For comparisons between proportions, the Fisher's Exact Test or Pearson Chi-Square test will be used as appropriate. Differences with a p-value < 0.05 will be regarded as statistically significant. For correlation analysis, the appropriate Chi-Square tests of association with confidence intervals will be conducted.

Multivariate/bivariate analyses will be conducted to assess relationships between demographic variables and measures of substance use. For multivariate analysis of dichotomous dependent variables, multivariate comparisons will be carried out using logistic regression. Odds Rations (OR) or T-tests with 95% confidence intervals will be reported for variables found to be significant in multivariate analysis. These are generated from the Logistic Regression process. Analysis of covariance (ANOVA) will also be used to examine the relationship between data variables and detected substance concentrations and types.

6.1 Proposed Research Time Frame

Year		2015																																			
Month		April				May				June				July				August				September				October				November				December			
Week		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
No.	ACTIVITY																																				
1	Writing Research Proposal																																				
2	Literature Review																																				
3	Ethics Approval																																				
4	Induction of mortuary-related procedures, Communication with Forensic Pathologists and Forensic Pathology Officers, Training in the Pharmacology/Toxicology Laboratory																																				
5	Specimen Sampling																																				
6	Toxicology Experiments																																				
7	Data Analysis and Interpretation																																				
8	Dissertation Write-up																																				

6.2 *Proposed Budget*

Supplied by:	Items	Amount (Rands)
UCT Division of Forensic Medicine	**Consumables:** • Grey and Red top 10mL tubes	R 1800
	• Markers	R 32
	• Self-stick labels	R 80
	• Large plastic containers for storage of samples	R 800
	• Evidence bags	R 0 (already acquired)
	• Blue top tubes	R 0 (already acquired)
	Administrative: • Printing & Photocopying	R 500
UCT Division of Clinical Pharmacology	**All the laboratory bench work and supplies**	R 10 000
TOTAL =		**R 13 212**

Ethical Considerations

The procedures used followed on from, and are covered by the clinical pharmacology registered database HREC No. R016/2014.

In this study, biological specimens will be obtained during autopsy of HOM, SUI, ACC, and UND cases from the SRM. The specimen collection does present any physical harm or health risk to the individuals. Additionally, the procedures of bio-sample collection, analysis, reporting and interpretation form part of the routine toxicological investigation, which falls under the package of care for post-mortem examinations delivered by the FPS. This toxicological analysis would usually assists the forensic pathologists' medico-legal investigation on the determination of cause of death.

The confidentiality of the study will be maintained during the whole study period. The information obtained from the mortuary death cases will remain anonymous and will be stored in a restricted access database at UCT. The specimen containers will be labelled with an assigned identification number specific to this study to ensure patient samples' anonymity, and stored in a controlled access refrigerator in the Clinical Pharmacology Laboratory.

This research project has obtained permission from Professor Lorna J Martin, the Head of the Division of Forensic Medicine, for the collection of biological samples from deceased persons at the SRM. Likewise, approval/clearance by the UCT Human Research Ethics Committee will be obtained before commencement.

Benefits of the Study

This research study will provide information to the South African criminological and forensic communities on the use of toxicological investigations as a tool for evaluating community-level drug use and fatalities.

Further, this study would provide essential and reliable qualitative descriptive toxicological data on the prevalence of substance abuse in fatalities common to Cape Town. This project will demonstrate the necessity for routine sample collection and toxicological analyses of post-mortem samples, especially for cases such as those studied during this research.

This study would be beneficial to the local community by providing information that can be used for substance abuse awareness and policy development on drug use and violence. The results of this study can be used to develop measures to reduce drug use and associated acts of violence, as well as to set up targeted prevention initiatives on drug use and abuse.

To future researchers, this study can provide important baseline information on the dynamics between drugs of abuse and violent injury deaths in South Africa.

References

1. World Health Organization (WHO). Global status report on violence prevention 2014. [Accessed 2016 Jan 10]. Available from: http://www.who.int/violence_injury_prevention/violence/status_report/2014

2. World Health Organization (WHO). Injuries and Violence: The Facts 2010. Geneva, Switzerland. [Accessed 2016 Jan 5]. Available from: http://www.who.int/violence_injury_prevention/en/

3. Dada MA, McQuoid-Mason DJ. Introduction to Medico-legal Practice. Durban: Butterworths; 2001.

4. World Health Organization (WHO). Global Health Observatory Data Repository: Cause of death 2008 summary tables. 2008 [Accessed 2015 Apr 7]. Available from: www.who.int

5. Bartolomeos K, Kipsaina C, Grills N, Ozanne-Smith J, Peden M (eds). Fatal injury surveillance in mortuaries and hospitals: a manual for practitioners. Geneva: World Health Organization; 2012.

6. Krug EG, Dahlberg LL, Mercy JA, Zwi AB, Lozano R (eds). World report on violence and health. Geneva, World Health Organization, 2002.

7. Bradshaw D, Nanna N, Laubscher R, Groenewald P, Joubert J, Nojilana B, et al. South African National Burden of Disease Study 2000: Estimates of Provincial Mortality. Cape Town; 2004.

8. Matzopoulos R, Norman R, Bradshaw D. The burden of injury in South Africa: Fatal injury trends and international comparisons. In: Suffla S, Van Niekerk A, Duncan N, editors. Crime, Violence Inj. Prev. South Africa Dev. Challenges. Medical Research Council- University of South Africa; 2004. p. 9–21.

9. Seedat M, Van Niekerk A, Jewkes R, Suffla S, Ratele K. Violence and injuries in South Africa: prioritising an agenda for prevention. Lancet (London, England). 2009;374:1011–22.

10. South African Medical Research Council. A profile of fatal injuries in South Africa: Tenth annual report (2008) of the National Injury Mortality Surveillance System. Pretoria, South Africa; 2010.

11. South African Medical Research Council. A profile of fatal injuries in South Africa: Nineth annual report (2007) of the National Injury Mortality Surveillance System. Pretoria, South Africa; 2008.

12. Groenewald P, Berteler M, Bradshaw D, Coetzee D, Cornelius K, Daniels J, et al. Western Cape Mortality Profile 2010. Cape Town: Cape T. South African Med. Res. Counc. 2013. Cape Town; 2013.

13. United Nations Office on Drugs and Crime. World Drug Report 2014 (United Nations publication, Sales No. E.14.XI.7).

14. UNODC. World Drug Report 2011 (United Nations Publication, Sales No. E.11.XI.10).

15. Dada S, Town C, Burnhams NH, Town C, Williams Y, Town C, et al. Monitoring Alcohol and Drug Abuse Treatment Admissions in South Africa. Cape Town, South Africa; 2013.

16. Wechsberg WM, Luseno WK, Karg RS, Young S, Rodman N, Myers B, et al. Alcohol, cannabis, and methamphetamine use and other risk behaviours among Black and Coloured South African women: a small randomized trial in the Western Cape. Int. J. Drug Policy. 2008;19:130–9.

17. City of Cape Town (2014). State of Cape Town Report 2014.

18. Kapp C. Crystal meth boom adds to South Africa's health challenges. Lancet (London, England). 2008;371:193–4.

19. Boles SM, Miotto K. Substance abuse and violence: A review of the literature. Aggress. Violent Behav. 2003;8:155–74.

20. Parker RN, Auerhahn K. Alcohol, Drugs, and Violence. Annu. Rev. Sociol. 1998;24:291–311.

21. Darke S, Duflou J. Toxicology and circumstances of death of homicide victims in New South Wales, Australia 1996-2005. J. Forensic Sci. 2008;53:447–51.

22. Darke S, Duflou J, Torok M. Drugs and violent death: Comparative toxicology of homicide and non-substance toxicity suicide victims. Addiction. 2009;104:1000–5.

23. Room R, Rossow I. The share of violence attributable to drinking. J. Subst. Use. 2001;6:218–28.

24. Darke S, Degenhardt L, Mattick R. Mortality amongst Illicit Drug Users: Epidemiology, Causes and Intervention. Cambridge: Cambridge University Press; 2006.

25. UNODC Global Study on Homicide 2013 (United Nations publication, Sales No. 14.IV.1).

26. Galea S, Ahern J, Tardiff K, Leon AC, Vlahov D. Drugs and firearm deaths in New York City, 1990-1998. J. Urban Health. 2002;79:70–86.

27. Gill JR, Catanese C. Sharp injury fatalities in New York City. J. Forensic Sci. 2002;47:554–7.

28. Gill JR, Lenz K a, Amolat MJ. Gunshot fatalities in children and adolescents in New York City. J. Forensic Sci. 2003;48:832–5.

29. Tardiff K, Marzuk PM, Leon AC, Hirsch CS, Stajić M, Portera L, et al. Cocaine, opiates, and ethanol in homicides in New York City: 1990 and 1991. J. Forensic Sci. 1995;40:387–90.

30. Tardiff AKJ, Wallace Z, Tracy M, Piper TM, Vlahov D, Galea S. Drug and alcohol use as determinants of New York City homicide trends from 1990 to 1998. J. Forensic Sci. 2005;50:470–4.

31. Hedlund J, Ahlner J, Kristiansson M, Sturup J. A population-based study on toxicological findings in Swedish homicide victims and offenders from 2007 to 2009. Forensic Sci. Int. 2014;244:25–9.

32. Harris EC, Barraclough B. Suicide as an outcome for mental disorders. A meta-analysis. Br. J. Psychiatry. 1997;170:205–28.

33. Darke S, Duflou J, Torok M. Toxicology and circumstances of completed suicide by means other than overdose. J. Forensic Sci. 2009;54:490–4.

34. Wilcox HC, Conner KR, Caine ED. Association of alcohol and drug use disorders and completed suicide: an empirical review of cohort studies. Drug Alcohol Depend. 2004;76 Suppl:11–9.

35. Centers for Disease Control and Prevention (CDC). Toxicology testing and results for suicide victims--13 states, 2004. MMWR. Morb. Mortal. Wkly. Rep. 2006;55:1245–8.

36. Crombie IK, Pounder DJ, Dick PH. Who takes alcohol prior to suicide? J. Clin. Forensic Med. 1998;5:65–8.

37. Dhossche DM, Rich CL, Ghani SO, Isacsson G. Patterns of psychoactive substance detection from routine toxicology of suicides in Mobile, Alabama, between 1990 and 1998. J. Affect. Disord. 2001;64:167–74.

38. Marzuk PM, Tardiff K, Leon AC, Hirsch CS, Stajic M, Hartwell N, et al. Use of prescription psychotropic drugs among suicide victims in New York City. Am. J. Psychiatry. 1995;152:1520–2.

39. Shields LBE, Hunsaker DM, Hunsaker JC, Ward MK. Toxicologic findings in suicide: a 10-year retrospective review of Kentucky medical examiner cases. Am. J. Forensic Med. Pathol. 2006;27:106–12.

40. Rich CL, Dhossche DM, Ghani S, Isacsson G. Suicide methods and presence of intoxicating abusable substances: some clinical and public health implications. Ann. Clin. Psychiatry. 1998;10:169–75.

41. Ohberg A, Vuori E, Ojanperä I, Lonngvist J. Alcohol and drugs in suicides. Br. J. Psychiatry. 1996;169:75–80.

42. Coklo M, Stemberga V, Cuculic D, Sosa I, Bosnar A. Toxicology and methods of committing suicide other than overdose. Med. Hypotheses. 2009;73:809–10.

43. Mathers C, Fat DM, Boerma JT. The global burden of disease: 2004 update. Geneva, Switzerland: World Health Organization (WHO); 2008 [Accessed 2015 Nov 2]. Available from: www.who.int/evidence/bod

44. Warner M, Chen LH, Makuc DM, Anderson RN, Miniño AM. Drug poisoning deaths in the United States, 1980-2008. NCHS Data Brief. 2011;1–8.

45. Breiding MJ, Wiersema B. Variability of undetermined manner of death classification in the US. Inj. Prev. 2006;12 Suppl 2:ii49–54.

46. Donaldson AE, Larsen GY, Fullerton-Gleason L, Olson LM. Classifying undetermined poisoning deaths. Inj. Prev. 2006;12:338–43.

47. Western Cape Department of Health. Forensic Pathology Service. [Accessed 2015 Apr 7]. Available from: www.westerncape.gov.za

48. Parry CD, Plüddemann A, Louw A, Leggett T. The 3-metros study of drugs and crime in South Africa: findings and policy implications. Am. J. Drug Alcohol Abuse. 2004;30:167–85.

49. Thomson JDS. Coloured homicide trends in South Africa. South African Crime Q. 2004;9–14.

50. Peden M, Van Der Spuy J, Smith P, Bautz P. Substance abuse and trauma in Cape Town. South African Med. J. 2000;90:251–5.

51. Lennings CJ, Copeland J, Howard J. Substance use patterns of young offenders and violent crime. Aggress. Behav. 2003;29:414–22.

52. Friedman AS. Substance use/abuse as a predictor to illegal and violent behavior. Aggress. Violent Behav. 1998;3:339–55.

53. Wagner EF. Substance use and violent behavior in adolescence. Aggress. Violent Behav. 1996;1:375–87.

54. Moffat AC, Osselton D, Widdop B, Clarke EGC. Clarke's Analysis of Drugs and Poisons: In Pharmaceuticals, Body Fluids and Postmortem Material. 3rd ed. Pharmaceutical Press; 2004.

55. Tytgat J. Screening of Urine and Blood Using Limited Sample Preparation and Information Dependent Acquisition with LC-MS/MS as Alternative for Immunoassays in Forensic Toxicology. J. Forensic Toxicol. Pharmacol. 2013;02.

56. Deventer K, Pozo OJ, Verstraete AG, Van Eenoo P. Dilute-and-shoot-liquid chromatography-mass spectrometry for urine analysis in doping control and analytical toxicology. TrAC Trends Anal. Chem. 2014;55:1–13.

57. ABSciex. A Rapid iMethod™ Test for Drugs of Abuse Screening (2010) [Accessed 2016 Apr 15]. Available from: www.absciex.com

Appendices

List of Appendices

Appendix A: **Post-Mortem Toxicology Sample Collection Form**

Post-Mortem Toxicology

Appendix A : Sample Collection Form

SECTION A : GENERAL CASE INFORMATION					
Date		Death Case Category			
Ref WC No.	WC/11/	HOM	☐	SUI	☐
Case ID		ACC	☐	UND	☐

SECTION B : DECEDENT INFORMATION					
Age				Date and Day of Death	
Race				Time of Death	
Sex	Male		Female	Area of Death	
Body Location: (e.g. Open Area, Water, Structure, Vehicle)				History (social/drug use):	

SECTION C : HOSPITALIZATION-RELATED HISTORY						
Was the deceased hospitalized before his/her death?	Yes		No			
If yes, in which hospital?	What was the length of hospitalization?					
Were toxicological analysis performed on blood in hospital?	Yes		No		N/A	
If yes, please list the results:						
Were any drugs administered during admission in hospital?	Yes		No		N/A	
If yes, please list drugs:						

SECTION D : DEATH SCENE HISTORY						
Circumstances of Death: (e.g. stabbing, assault, shooting, hanging, strangulation, suffocation, other)						
Complete where relevant	*Stabbing*	*Assault*	*Shooting*	*HSS*	*Other*	*N/A*
Weapon/s present on scene (tick if yes)						

1

Alleged/Type of weapon/s (e.g. gun, knife)						
Ligature or device present on scene (tick if yes)						
Description of ligature						
Additional Comments						

SECTION E : AUTOPSY INFORMATION							
Date of Autopsy		**Date of Specimen Collection**		**Date of Death**			
Suspected manner of death	Homicide		Suicide		Accident		Undetermined
Cause of Death							
Additional Comments on Case History (e.g. drug paraphernalia on scene and/or body, social history):							
Significant Post-Mortem Observations by Pathologist:							

SECTION F : SPECIMENS COLLECTION

Sample Type	*ID No's*	*Volume (ml)*	*Condition/Appearance*
Peripheral Blood			
Heart Blood			
Urine			
Vitreous Humor			
Bile			
Hair			
Other, specify:			
Seal Bag No.			
Additional Comments on Specimen Collection:			

SECTION G : OFFICIAL RECEIPT COMFIRMATION

Forensic Pathology Services (FPS):

I hereby authorise the custody of these samples to:

______________________ ______________________

Student: Kathrina Auckloo (ACKMAR005) **Forensic Pathologist**

______________________ ______________________

DD/MM/YY **DD/MM/YY**

Appendix B: **Post-Mortem Toxicology Specimen Submission Form**

Post-Mortem Toxicology

Appendix B : Specimen Submission Form

SECTION A : GENERAL CASE INFORMATION	
Date	
Case ID	
Seal Bag No.	

SECTION B : SPECIMENS COLLECTION

Sample Type	*ID No.*	*Volume (ml)*	*Condition/Appearance*
Peripheral Blood			
Heart Blood			
Urine			
Vitreous Humor			
Bile			
Hair			
Other, specify:			
Seal Bag No.			
Additional Comments on Specimen Collection:			

The specimens indicated above were labelled and packaged in the corresponding evidence bag, and are being secured at the Toxicology Laboratory in Groote Schuur Hospital, Cape Town by student *Kathrina Auckloo (ACKMAR005)*, where the samples will be analysed by *Kathrina Auckloo (ACKMAR005)* for the presence of drugs of abuse.

SECTION B : OFFICIAL RECEIPT COMFIRMATION

Forensic Pathology Services (FPS):

I hereby authorise the order of the analysis by the student:

Student: Kathrina Auckloo (ACKMAR005)

Toxicology Laboratory Personnel

Appendix C: **Drugs of Abuse Screening Method** [57].

APPENDIX C

A Rapid iMethod™ Test for Drugs of Abuse Screening

iMethod™ Test for Drugs of Abuse Screening Version 2.0 for Cliquid® Software

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC/MS/MS) has quickly become the technique of choice for both screening and confirmation due to elimination of derivatization and simplification of sample preparation, while offering better sensitivity and selectivity, shorter run times, and the ability to analyze wider polarities and molecular weight range of compounds in a single analysis.

The following description outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the Screening of Drugs of Abuse in plasma, blood or urine when using an AB SCIEX 3200 QTRAP® instrument. The use of this instrument allows the utilization of an information dependent acquisition consisting of an MRM survey scan that triggers product ion dependent scans that can be used for compound confirmation via library searching. This method has also been developed and verified for use with 4000 QTRAP® LC/MS/MS system. This iMethod™ Test includes both an iMethod™ test for the screening of 700 drugs of abuse in a single run as well as an MRM catalogue containing over 1250 drugs of abuse and related compounds, with up to 6 transitions per compound. The MRM catalogue can be used to create additional custom screening and/or quantification methods. Please note that the AB SCIEX 1250 compound forensic drug library required for confirmation is sold separately.

A number of example sample preparation procedures are provided, based upon the matrix, the degree of clean up required and/or the preferred mode of ionization. These range from a simple extraction and dilution for urine to liquid-liquid for blood and plasma to solid phase extraction for plasma and urine. Internal standards of Diazepam-D_5 and Doxepin-D_3 at known concentration are added during sample preparation to monitor recovery.

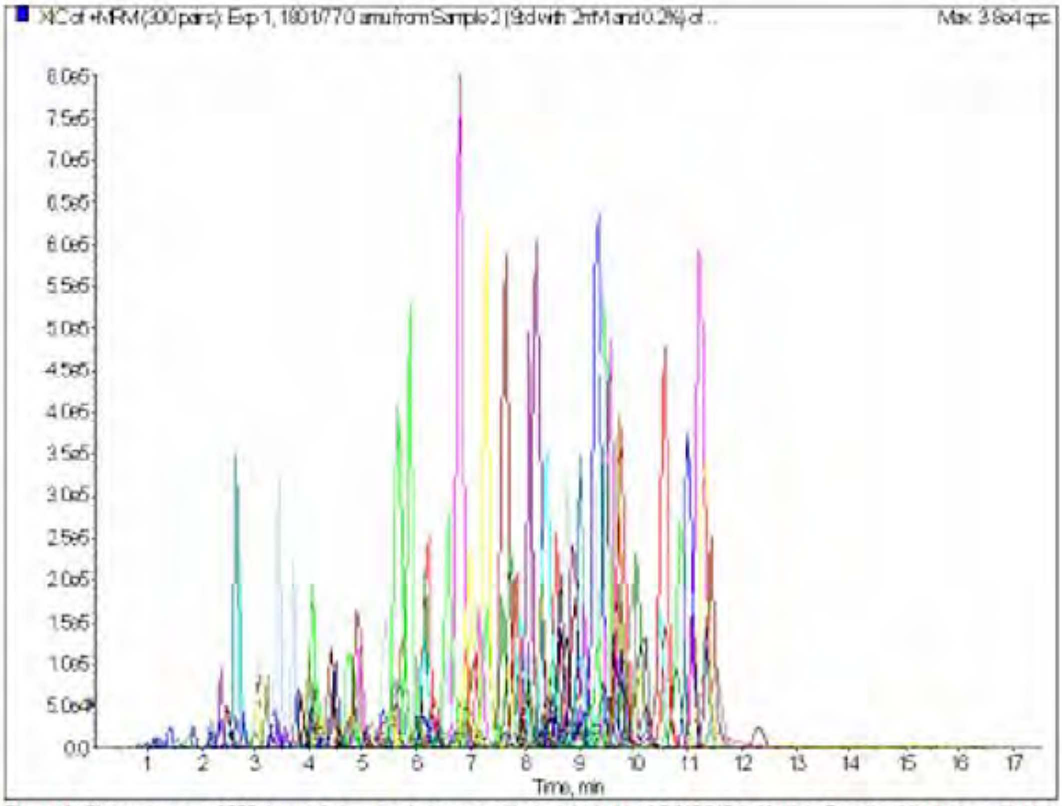

Figure 1: Chromatogram of 300 drugs of abuse and pharmaceuticals detected by LC/MS/MS in Multiple Reaction Monitoring with highest selectivity and sensitivity

p 1

Table 1: List of compounds in the 700 compound drug screening method.

Compound name	Compound name	Compound name	Compound name
17-alpha-Methyltestosterone	Amiodarone	Bisoprolol	Cimetidine
2-Amino-5-chlorobenzophenone	Amiphenazole	Bomaprine	Cinnarizine
2-Amino-5-nitrobenzophenone	Amisulpride	Brodifacoum	Cinoxacin
2-Hydroxyethylflurazepam	Amitriptylin	Bromacil	Ciprofloxacin
3,4-Methylenedioxyamphetamine	Amitrole	Bromazepam	Cisapride
3,4-Methylenedioxyethylamphetamine	Amlodipine	Bromocriptine	Citalopram
3,4-Methylenedioxymethamphetamine	Ammoidin	Brompheniramine	Clarithromycin
3,5-Diiodotyrosine	Amorolfine	Brotizolam	Clemastine
4-Benzamidosalicyclic acid	Amoxicillin	Bucetin	Clenbuterol
6-Mercaptopurine	Amphetamine	Bufexamac	Clobazam
6-O-Monoacetylmorphine	Amrinone	Bumetanide	Clobenzepam
7-Aminoclonazepam	Antazoline	Bunazosin	Clobutinol
7-Aminodesmethylflunitrazepam	Apalcillin	Bunitrolol	Clomethiazole
7-Aminoflunitrazepam	Apomorphine	Bupivacaine	Clomipramine
7-Aminonitrazepam	Apophedrin	Bupranolol	Clonazepam
9-Hydroxyrisperidone	Apraclonidin	Buprenorphine	Clonidine
Acebutolol	Aprindine	Buprofezin	Clopenthixol
Aceclidine	Atenolol	Buspirone	Clopidogrel
Aceclofenac	Atorvastatin	Butaperazine	Cloprednol
Acemetacin	Atraton	Butetamate	Clozapine
Acepromazine	Atrazine-desethyl	Butoxycaine	Cocaine
Acepromethazine	Atropine	Cafaminol	Codeine
Acetamin	Atropinmethylbromid	Caffeine	Coniine
Acetylaminonitroprophoxybenzene	Azatadine	Candesartan	Corticosterone
Aciclovir	Azelastine	Caproylresorcinol	Cortisone
Actinoquinol	Azintamid	Captopril	Cotinin
Adenine	Aztreonam	Carazolol	Coumatetralyl
Adrenalone	Baclofen	Carbamazepine	Croconazole
Ajmaline	Bambuterol	Carbamazepine 10,11-epoxide	Cromoglicicacid
Alachlor	Bamifylline	Carbendazim	Cyamemazine
Alclometasone-17,21-dipropionate	Bamipin	Carbinoxamine	Cyclicine
Alimemazine	Beclamide	Carbutamide	Cyclophosphamide
Alizapride	Beclometasone dipropionate	Carbuterol	Cyclovalone
Allopurinol	Befunolol	Carteolol	Cyproheptadine
Almitrine	Bendiocarb	Carvedilol	Cytarabine
alpha-Hydroxyalprazolam	Benfluorex	Celiprolol	D3-Doxepin
alpha-Hydroxytriazolam	Benodanil	Cerivastatin	D5-Diazepam
Alprazolam	Benperidole	Cetirizine	Dapiprazole
Alprenolol	Benproperine	Chinine	Deflazacort
Altretamine	Bensultap	Chlorazanil	Demeton-S-methylsulfone
Alypin	Bentiromide	Chlorbenzoxamine	Denaverine
Amantadine	Benzatropine	Chlorcyclizine	Desalkylflurazepam
Ambroxol	Benzocaine	Chlordiazepoxide	Desipramine
Ambucetamide	Benzoctamine	Chloridazon	Desmedipham
Amezinium	Benzododecinium	ChlormadinoneAcetate	Desmethylclobazam
Amidopyrin	Benzoylecgonine	Chlormequat	Desmethylclomipramine
Amidotrizoic Acid	Benzthiazuron	Chlorphenethiazine	Dexamethasone
Amiloride	Berberine	Chlorpheniramine	Dexamethasone 21-isonicotinate
Aminodantrolene	Betamethasone 21-phosphate	Chlorpromazine	Dexfenfluramine
Aminoglutethimide	Betaxolol	Chlorpromazine Sulfoxide	Dextromethorphan
Aminophenazone	Bevonium	Chlorprothixene	Dextropropoxyphene
Aminopromazine	Bezafibrate	Cilastatin	Diaveridine
Aminorex	Biperiden	Cilazapril	Diazepam

AB SCIEX

Compound name	Compound name	Compound name	Compound name
Dibutyladipate	Fenpipramide	Hydrocortisone	Mefenorex
Diclofenac	Fenpiprane	Hydrocortisone 21-acetate	Mefexamide
Dicycloverine	Fenproporex	Hydromorphone	Mefloquine
Dienogest	Fentanyl	Hydroxyzine	Melatonin
Diethazine	Fenticonazole	Hymecromone	Melitracen
Diethylcarbamazine	Fexofenadine	Imipramine	Meloxicam
Difenoconazole	Flecainide	Indanazoline	Melperone
Difenoxuron	Flocoumafen	Indapamine	Melphalan
Diflucortolone	Floctafenine	Indinavir	Mepindolol
Dihydrocodeine	Fluanisone	Indoprofen	Mepivacaine
Dihydroergocristin	Fluconazole	Indoramin	Meptazinol
Dihydroergotamine	Flumazenil	Iopodicacid	Mequitazine
Dilazep	Flunarizine	Ipratropium	Mescaline
Diltiazem	Flunitrazepam	Iprazochrome	Mesoridazine
Dimetindene	Fluoxetine	Irbesartan	Mesuximide
Dimetotiazine	Flupentixol	Isoaminile	Metaclazepam
Dimetridazole	Fluphenazine	Isoconazole	Metamfepramone
Dioxethedrin	Flupirtine	Isoprenaline	Metamitron
Diphenamid	Flurazepam	Isoproturon	Metamphetamine
Diphenhydramine	Flurochloridone	Isothipendyl	Metazachlor
Diponium	Fluspirilen	Isoxicam	Metenolone acetate
Diprophylline	Fluticasone Propionate	Isoxsuprine	Metformin
Dipyridamole	Fluvastadine	Kavain	Methabenzthiazuron
Disopyramide	Fluvoxamine	Ketamine	Methadone
Dixyrazine	Fuberidazole	Ketoprofen	Methaphenilene
Dobutamine	Furalaxyl	Ketorolac	Methapyrilene
Dorzolamide	Gabapentin	Ketotifen	Methaqualon
Doxapram	Galantamine	Lamotrigine	Methazolamide
Doxepin	Gallopamil	Lercanidipine	Methfuroxam
Doxylamine	Gemcitabine	Levocabastine	Methocarbamol
Drofenine	Glibenclamide	Levodopa	Methoprotryne
Ecgoninemethylester	Glibornuride	Levomepromazine	Methotrexate
EDDP	Gliclazide	Levopropylhexedrin	Methylephedrine
Embutramide	Glimepiride	Lidocaine	Methylphenidate
Enalapril	Glipizide	Lisinopril	Methylscopolamine
Enoximone	Gliquidone	Lonazolac	Methylthiouracil
Ephedrine	Griseofulvin	Loperamide	Metipranolol
Eprosartan	Guaifenesin	Loratadine	Metixene
Esculin	Guanabenz	Lorazepam	Metoprolol
Esmolol	Guanethidine	Lormetazepam	Metronidazole
Estazolam	Guanfacine	Losartan	Metsulfuron-methyl
Ethenzamide	Guanoxan	Loxapine	Metyrapone
Etofenamate	Haloperidol	Lysergide	Mexiletine
Etomidate	Haloxyfop ethoxyethyl ester	Mafenide	Mianserin
Etoposide	Heptaminol	Maprotiline	Miconazole
Famotidine	Heroin	MBDB	Midazolam
Fedrilate	Hexazinone	Mebeverine	Midodrine
Felbamate	Hexobendine	Meclizine	Milrinone
Felodipine	Histamine	Meclofenamic acid	Minoxidil
Fenarimol	Histapyrrodine	Mecloxamine	Mirtazapine
Fendiline	Homatropine	Meclozine	Mizolastine
Fenetylline	Hordenine	Medazepam	Moclobemide
Fenfluramine	Hydrocodone	Mefenamic acid	Modafinil
Molsidomine	Ondansetron	Pirbuterol	Ritodrine

Compound name	Compound name	Compound name	Compound name
Monocrotophos	Opipramol	Pirenzepine	Rizatriptan
Monolinuron	Orciprenaline	Piretanide	Ropinirole
Moperone	Ornidazole	Piritramide	Ropivacaine
Morphine	Orphenadrine	Piroxicam	Rosiglitazon
Morphine-3-β-D-glucuronide	Oxadixyl	Pizotifen	Salbutamol
Moxaverine	Oxamyl	Practolol	Salicylamide
Moxisylyte	Oxatomide	Prajmalium	Scopolamine
Moxonidine	Oxazepam	Pramipexole	Serotonin
Nabumetone	Oxcarbazepine	Prazepam	Sertindole
Nadolol	Oxeladin	Prazosin	Sertraline
Naftidrofuryl	Oxetacaine	Prednisolone	Sildenafil
Naftifine	Oxiconazole	Prednisone	Simazine
Nalidixic acid	Oxilofrine	Prilocaine	Sotalol
Nalorphine	Oxitriptan	Primaquine	Spirapril
Naloxone	Oxitropium	Primidone	Stanozolol
Naltrexone	Oxomemazine	Procainamide	Sulfabenzamide
Nandrolone	Oxprenolol	Prochlorperazine	Sulfadiazine
Naphazoline	Oxybuprocaine	Procyclidine	Sulfadoxine
N-Despropylpropafenone	Oxybutynin	Progesterone	Sulfaethidole
Nebivolol	Oxycodone	Promazine	Sulfaguanidine
Nefopam	Oxyfedrine	Promethazin	Sulfamethizole
Nicardipine	Oxymetazoline	Prometryn	Sulfamethoxazole
Nicotinamide	Oxymorphon	Propafenone	Sulfapyridine
Nicotine	Oxypertine	Propiconazole	Sulfaquinoxaline
Nifedipine	Papaverine	Propionylpromazine	Sulindac
Nifenazone	Paracetamol	Propipocaine	Sulpiride
Niflumic acid	Paraoxon	Propiverine	Sultiame
Nimodipine	Paroxetine	Propranolol	Sumatriptan
Nimorazole	Penfluridol	Propyphenazone	Suxibuzone
Nisoldipine	Pentamidine	Prothipendyl	Tadalafil
N-Isopropylsalicylamide	Pentoxyverine	Protriptyline	Talinolol
Nitrazepam	Perazine	Pseudoephedrine	Tamoxifen
Nitrendipine	Periciazine	Psilocin	Telmisartan
Nizatidine	Perindopril	Pyranocoumarin	Temazepam
N-Methylephedrine	Perphenazine	Pyribenzamine	Tenocixam
Nomifensine	Pethidine	Pyridoxine	Terazosine
Norbuprenorphine	Phenacetin	Pyrimethamine	Terbinafine
Nordiazepam	Phenazone	Pyrithiol	Terbutaline
Nordiazepam N-ethyl	Phenazopyridine	Pyrvinium	Terbutryn
Norephedrine	Phencyclidine	Quetiapine	Terconazole
Norethisterone	Phenelzine	Quinapril	Terfenadine
Norethisterone acetate	Pheniramine	Quinine	Tertatolol
Norfenefrine	Phenprocoumon	Ramipril	Tetracaine
Norfentanyl	Phenylephrine	Ranitidine	Tetrazepam
Norfloxacin	Phenyltoloxamine	Raubasine	Tetroxoprim
Normorphine	Phenytoin	Reboxetine	Tetryzoline
Nortriptyline	Pholedrine	Remoxipride	THC
Noscapine	Physostigmine	Repaglinide	THC-COOH
Nuarimol	Pilocarpine	Reproterol	THC-OH
Obidoxime	Pindolol	Reserpine	Thebacon
Ofloxacin	Pioglitazone	Riluzole	Theobromine
Olanzapine	Pipamperone	Risperidone	Theophylline
Thiabendazole	Tocainide	Triazolam	Urapidil
Thiamazole	Tolazoline	Trifluperazine	Valdecoxib

Compound name	Compound name	Compound name	Compound name
Thiamine	Tolbutamide	Trifluperidol	Valsartan
Thiopropazate	Toliprolol	Triflupromazine	Vardenafil
Thioproperazine	Tolnaftate	Trimethobenzamide	Venlafaxine
Thioridazine	Tolpropamine	Trimethoprim	Verapamil
Thiram	Tolycaine	Trimipramine	Vincamine
Tiagabine	Topotecan	Tripelennamine	Warfarin
Tiapride	Torasemide	Triprolidine	Xantinol
Ticlopidine	Tramadol	Tritoqualine	Xipamide
Tiemonium	Tranexamic acid	Tromantadine	Xylometazoline
Tilidine	Trazodone	Tropisetron	Yohimbine
Timolol	Triadimenol	Trospium	Zolpidem
Tinidazole	Triallate	Tryptamine	Zopiclone
Tizanidine	Triamterene	Tulobuterol	Zuclopenthixol

Results

An example chromatogram for drugs of abuse and screening is shown in Figure 1 to highlight the possibility of detecting hundreds of compounds in a single experiment. An example reports generate automatically after analysis by Cliquid® Software is presented in Figure 2.

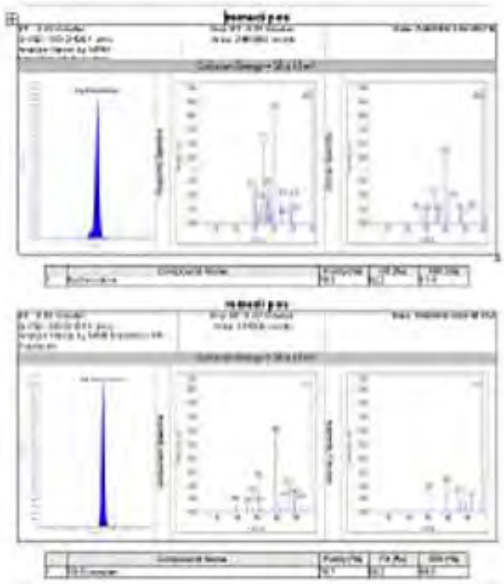

Figure 2: Example screening report.

System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 3200 QTRAP® or 4000 QTRAP® LC/MS/MS System
- A Shimadzu Prominence 20A LC System with reservoir tray and bottles, system controller CBM-20A, 100 µL mixer, 2 isocratic pumps LC-20AD, 3 channel degasser Autosampler SIL-20AC, column oven CTO-20AC or Agilent 1100/1200 LC system with binary pump G1312A (without static mixer), well plate auto sampler, and thermostated column oven.
- LC/MS grade water, acetonitrile, ammonium formate and formic acid
- 1.5 mL Eppendorf tubes
- A Restek pre-column, 5µm 60Å, PFP Propyl, 10 x 2.1 mm
- A Restek analytical column, 5µm 60Å, PFP Propyl Column, 50 x 2.1 mm (included in the iMethod Test)
- SPE cartridges, 200mg Chromabond® Macherey-Nagel
- A centrifuge able to accommodate Eppendorf tubes and run at 14000 rpm
- Pipettes and standard laboratory glassware

Ordering Information

Product Name	Part Number
iMethod™ Test for Drugs of Abuse Screening Version 2.0 for Cliquid® Software	1040049

While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the Analysis of Drugs of Abuse, please note that the results obtained do require some experience with LC/MS/MS and sample preparation procedures. As such, web-based and on-site training are available to assist in the deployment of the iMethod™ Test and are recommended for inexperienced users. Please consult your local sales representative for more details.

Important Note

The iMethod™ Test described above has been designed by AB SCIEX to provide the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. This method is provided for information purposes only. The performance of this method is not guaranteed due to many different potential variations, including instrument performance, tuning, and maintenance, chemical variability and procedures used, technical experience, sample matrices, and environmental conditions. It us up to the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

The purchase and use of certain of the chemicals listed below may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements. AB SCIEX is not responsible for user's compliance with any statute or regulation, or for any permit or approval required for user to implement any iMethod™ procedure.

The information included in this product is intended for reference and research purposes only. AB SCIEX offers no guarantee as to the quality or suitability of this data and suitability of the information included in this (Library, database, etc.) for use with your specific application.

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Appendix D1: **Participant Information Sheet**

UNIVERSITY OF CAPE TOWN

APPENDIX D1

RESEARCH PROJECT - "A Post-Mortem Toxicological Investigation: Understanding the Role of Drugs of Abuse in Violent Fatalities in Cape Town, South Africa".

Division of Forensic Medicine and Toxicology, University of Cape Town

INFORMATION SHEET FOR RESEARCH PARTICIPANTS

Purpose of the Study:

Violence is a critical health and public safety issue in South Africa. Unfortunately, violence often results in the death of an individual. Our communities in the Western Cape also experience high substance (prescription/illicit) use and abuse. Everyone of any age, sex, race, economic or income status may be affected by substance abuse. It is important to provide information to families as to why the death may have occurred so as to assist in understanding and closure.

South Africa currently has limited to no information on substance use and violent injury deaths; however, a connection has been reported in international research. This research project aims to identify the incidence of substances in suspected violent fatalities and understand the role these substances may play in contributing to death, hoping to improve the management of violence-related fatalities.

This study will assist the process of death investigation in South Africa and provide essential data for future community support to reduce drug-use and related fatalities.

What will the study involve?

The study will involve testing for drugs in biological samples routinely collected during an autopsy from individuals who have passed away in a suspected violent manner. It will also include the anonymized collection of information of the alleged incident.

Samples will be collected during the routine autopsy procedure at the Salt River Mortuary. This process is routinely performed in unexpected or unnatural deaths as indicated in the Inquest Act of South Africa. No additional samples will be collected following the autopsy.

The name of the decedent will be removed from any information or samples that are collected, and no one will be able to identify them when looking at the study results.

All laboratory analyses will be conducted at the University Of Cape Town Clinical Pharmacology Laboratory at Groote Schuur Hospital. The data generated from the toxicological analysis of the bio-samples will be stored anonymously in a restricted access database at the University Of Cape Town. The information that we collect from this research project will be kept confidential and no one will be identifiable in any data produced from this study.

Why have you been asked to provide consent for the decedent's samples?

You have been asked to take part because you are presenting to the mortuary after a family member has passed away in a suspected violent manner. This study wants to look at the incidence of drugs (prescription/illicit) in our communities and understand their role in violence and fatalities. Please remember that the laboratory results are confidential and will not alter any criminal procedures or outcomes.

We need to know if violent fatalties are related to drug use in our community, in order for us to possibly assist in the management and prevention of such cases. In other countries, these cases are routinely tested for drugs and/or alcohol, but currently this is not performed in South Africa.

Do you have to take part?

No, your help/participation is entirely voluntary. You will be invited to participate, and if you agree and are willing, you can indicate this by signing the consent form. You can keep a copy of this information sheet and the consent form. You may withdraw your consent at any stage.

Your decision whether or not to participate will not affect the services normally provided to you by Forensic Pathology Services.

Will your participation in the study be kept confidential?

Yes. The decedent's name and details will be removed from the information and samples that we collect, and all results will be confidential and will not be shared with or given to anyone.

What will happen to the results?

The results of the study may be seen by all the researchers that form part of this study and the doctors who perform the postmortem. The results of the study may be published in a research journal, or presented at a conference. Confidential information will not be shared by the investigators in any capacity. This research will form the basis for future research in post-mortem forensic toxicology in South Africa, which will assist forensic professionals and in turn inform and aid the public.

Are there disadvantages of taking part?

We don't envisage any negative consequences for taking part in this study.

Who has reviewed this study?

Approval has been obtained by the Human Research Ethics Committee of the University of Cape Town, Faculty of Health Sciences.

Any further queries?

If you need any further information or have any additional queries please contact:

Bronwen B. Davies, Division of Forensic Medicine, Falmouth Building, Entrance 2, Level 5, Anzio Road, Observatory, Tel: 021-406-6026 or email: bronwen.davies@uct.ac.za

If you agree to take part in the study, please indicate this by signing the consent form overleaf.

Appendix D2: **Participant Information Sheet for Delayed Consent**

UNIVERSITY OF CAPE TOWN

APPENDIX D2

RESEARCH PROJECT - "A Post-Mortem Toxicological Investigation: Understanding the Role of Drugs of Abuse in Violent Fatalities in Cape Town, South Africa".
Division of Forensic Medicine and Toxicology, University of Cape Town

INFORMATION SHEET FOR RESEARCH PARTICIPANTS

Purpose of the Study:
Violence is a critical health and public safety issue in South Africa. Unfortunately, violence often results in the death of an individual. Our communities in the Western Cape also experience high substance (prescription/illicit) use and abuse. Everyone of any age, sex, race, economic or income status may be affected by substance abuse. It is important to provide information to families as to why the death may have occurred so as to assist in understanding and closure.

South Africa currently has limited to no information on substance use and violent injury deaths; however, a connection has been reported in international research. This research project aims to identify the incidence of substances in suspected violent fatalities and understand the role these substances may play in contributing to death, hoping to improve the management of violence-related fatalities.

This study will assist the process of death investigation in South Africa and provide essential data for future community support to reduce drug-use and related fatalities.

What will the study involve?
The study will involve testing for drugs in biological samples routinely collected during an autopsy from individuals who have passed away in a suspected violent manner. It will also include the anonymized collection of information of the alleged incident.

Samples will be collected during the routine autopsy procedure at the Salt River Mortuary. This process is routinely performed in unexpected or unnatural deaths as indicated in the Inquest Act of South Africa. No additional samples will be collected following the autopsy.

The name of the decedent will be removed from any information or samples that are collected, and no one will be able to identify them when looking at the study results.

All laboratory analyses will be conducted at the University Of Cape Town Clinical Pharmacology Laboratory at Groote Schuur Hospital. The data generated from the toxicological analysis of the bio-samples will be stored anonymously in a restricted access database at the University Of Cape Town. The information that we collect from this research project will be kept confidential and no one will be identifiable in any data produced from this study.

When does collection of toxicology samples occur?
In normal and ideal circumstances, the collection of samples takes place during the routine scheduled autopsy of the decedent following the informed consent interview and the body identification procedure by the decedent's next-of-kin.

However, sometimes, the scheduled autopsy procedure is performed before the body identification process and therefore before the informed consent interview with the family/relative. In such cases, the toxicological samples are collected prior to identification and informed consent interview, and stored according to routine protocols until delayed consent is obtained.

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In the event where obtaining delayed consent is unsuccessful, the respective samples will be destroyed and this will not affect the results of the autopsy procedure or have any negative consequence on the progress of this research study.

Why have you been asked to provide consent for the decedent's samples?
You have been asked to take part because you are presenting to the mortuary after a family member has passed away in a suspected violent manner. This study wants to look at the incidence of drugs (prescription/illicit) in our communities and understand their role in violence and fatalities. Please remember that the laboratory results are confidential and will not alter any criminal procedures or outcomes.

We need to know if violent fatalities are related to drug use in our community, in order for us to possibly assist in the management and prevention of such cases. In other countries, these cases are routinely tested for drugs and/or alcohol, but currently this is not performed in South Africa.

Do you have to take part?
No, your help/participation is entirely voluntary. You will be invited to participate, and if you agree and are willing, you can indicate this by signing the consent form. You can keep a copy of this information sheet and the consent form. You may withdraw your consent at any stage.
Your decision whether or not to participate will not affect the services normally provided to you by Forensic Pathology Services.

Will your participation in the study be kept confidential?
Yes. The decedent's name and details will be removed from the information and samples that we collect, and all results will be confidential and will not be shared with or given to anyone.

What will happen to the results?
The results of the study may be seen by all the researchers that form part of this study and the doctors who perform the postmortem. The results of the study may be published in a research journal, or presented at a conference. Confidential information will not be shared by the investigators in any capacity. This research will form the basis for future research in post-mortem forensic toxicology in South Africa, which will assist forensic professionals and in turn inform and aid the public.

Are there disadvantages of taking part?
We don't envisage any negative consequences for taking part in this study.

Who has reviewed this study?
Approval has been obtained by the Human Research Ethics Committee of the University of Cape Town, Faculty of Health Sciences.

Any further queries?
If you need any further information or have any additional queries please contact:

Bronwen B. Davies, Division of Forensic Medicine, Falmouth Building, Entrance 2, Level 5, Anzio Road, Observatory, Tel: 021-406-6026 or email: bronwen.davies@uct.ac.za

If you agree to take part in the study, please indicate this by signing the consent form overleaf.

Appendix E1: **Certificate of Consent**

UNIVERSITY OF CAPE TOWN

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APPENDIX E1: CERTIFICATE OF CONSENT

"A Post-Mortem Toxicological Investigation: Understanding the Role of Drugs of Abuse in Violent Fatalities in Cape Town, South Africa"

Part 1: Authorisation for collection of evidence and release of information

I, *(next-of-kin full name)*.. hereby consent to the collection of biological specimens from my familial decedent for toxicological laboratory analyses. I understand that this examination is carried out to assist in establishing the cause of death, a process which usually involves retention of tissue or samples for detailed laboratory examination. It has been explained to me that these tissues and samples, which are removed during the post-mortem, are of value to forensic toxicological research.

The purpose and nature of the study has been explained to me in writing.

I understand that participation is voluntarily.

I understand that I can withdraw from the study, without repercussions, at any time, whether before it starts or during participation.

I understand that anonymity will be ensured in the write-up by disguising my and the decedent's identity.

I voluntarily consent for the decedent's samples to be additionally used for this research.

Next-of-Kin Signature	Date
Attending Investigator Signature	Date

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UNIVERSITY OF CAPE TOWN

IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

Part 2: Retention and future use of tissue samples

Tissue samples and small amounts of bodily fluids taken during the autopsy for this research may be disposed of following the project completion. However, if permitted, these may be stored for use for future research. These samples can be extremely valuable for future toxicological research. The storage of the tissue/fluid samples and their later use requires your consent. Any additional research performed on stored samples will undergo full UCT Human Ethics review. Please indicate whether you consent to this (please tick if yes, leave blank if no):

☐ I consent to the fluid/tissue samples being stored for future use, and

☐ I consent to tissue/fluid samples being used for future research that has been approved by an appropriate ethics committee

________________________ ________________________

Next-of-Kin Signature Date

________________________ ________________________

Attending Investigator Signature Date

Study number: ………………

Appendix E2: **Certificate of Delayed Consent**

UNIVERSITY OF CAPE TOWN

APPENDIX E2: CERTIFICATE OF DELAYED CONSENT

"A Post-Mortem Toxicological Investigation: Understanding the Role of Drugs of Abuse in Violent Fatalities in Cape Town, South Africa"

Part 1: Authorisation for collection of evidence and release of information

I, *(next-of-kin full name)*,.. hereby consent to the collection of biological specimens from my deceased next-of-kin for laboratory analyses. I understand that this examination is carried out to [verify] the cause of death, a process which usually involves retention of tissue or samples for detailed laboratory examination. It has been explained to me that these tissues and samples, which are removed during the post-mortem, are of value to forensic toxicological research.

I confirm that:	*Yes*	*No*
• I have read and understood the contents of this form and give permission to assist in this research study.	☐	☐
• I have been informed about the purpose, procedures, possible benefits, and risks of the research study.	☐	☐
• I understand that I can withdraw my permission from the study, without repercussions, at any time, whether before it starts or during participation.	☐	☐
• I understand that routine toxicological samples were collected during the post-mortem examination of my deceased next-of-kin and stored according to standard protocols. I understand that these samples will be used for this research should I give permission.	☐	☐
• If I do not give permission, the toxicological samples collected will be discarded. I understand that this will not affect the results of the post-mortem examination or have any negative consequences whatsoever should I decided to not give permission.	☐	☐

____________________ Next-of-Kin Signature ____________________ Date

____________________ Attending Investigator Signature ____________________ Date

Page | 1

UNIVERSITY OF CAPE TOWN

Part 2: Retention and future use of tissue samples

Tissue samples and small amounts of bodily fluids taken during the autopsy for this research may be disposed of following the project completion. However, if permitted, these may be stored for use for future research. These samples can be extremely valuable for future toxicological research. The storage of the tissue/fluid samples and their later use requires your consent. Any additional research performed on stored samples will undergo full UCT Human Ethics review. Please indicate whether you consent to this (please tick if yes):

☐ I give permission for the storage of the deceased's fluid/tissue samples for future use, and

☐ I grant permission for future research that has been approved by an appropriate ethics committee using the deceased's tissue/fluid samples

Next-of-Kin Signature	Date
Attending Investigator Signature	Date

Study number: ....

Appendix F: **Amendments to Research Protocol**

Following the approval of the research proposal, the changes made in the study are listed below:

- The UND group, referring to 'undetermined deaths' was removed from the study after specimen collection as no cases of undetermined violent death were obtained during the study period.

- Due to technical complications during specimen collection such as waiting for ethics approval, introducing the project to the Salt River mortuary staff members, and training on collection methods with the forensic pathologists and forensic pathology officers, the duration of collection period was reduced from June-October 2015 (5 months) to August-October (3 months).

- Due to restrictions and challenges faced at the Salt River mortuary, as well as a shorter collection period, and the restricted duration of the whole project, the sample size was reduced from a minimum of 300 cases to 100 cases.

- The collection of liver and stomach contents was not performed due to limited time and resources.

- Hair was collected but not analysed for toxicology due to limited time and resources. This analysis of these specimens provide scope for a larger and expanded project in future.

Part B

Structured Literature Review

I. Introduction

Violence and resulting injuries constitute critical worldwide health and public safety issues, which greatly affect developing countries, such as that of South Africa. A violence-related injury can be understood as one inflicted by oneself or another person, on purpose or by using deliberate means. Fatalities due to violence-related injuries can result from a variety of causes such as gunshot, sharp and/or blunt force, drowning, burns, falls, bites and stings, and natural disasters amongst others. Violence- and injury-related fatalities are classified as being unnatural, and may further lie in one of four broad categories: homicide, suicide, accidental, and undetermined deaths. The abuse of psychoactive substances presents another significant global health problem, which is known to be associated with increased population morbidity and mortality [1]. Toxicological analysis in medico-legal investigations provides crucial information in determining the cause of death of an individual. Forensic researchers around the world are now using toxicology to better understand the magnitude of the involvement of licit and illicit drugs in violent deaths [2].

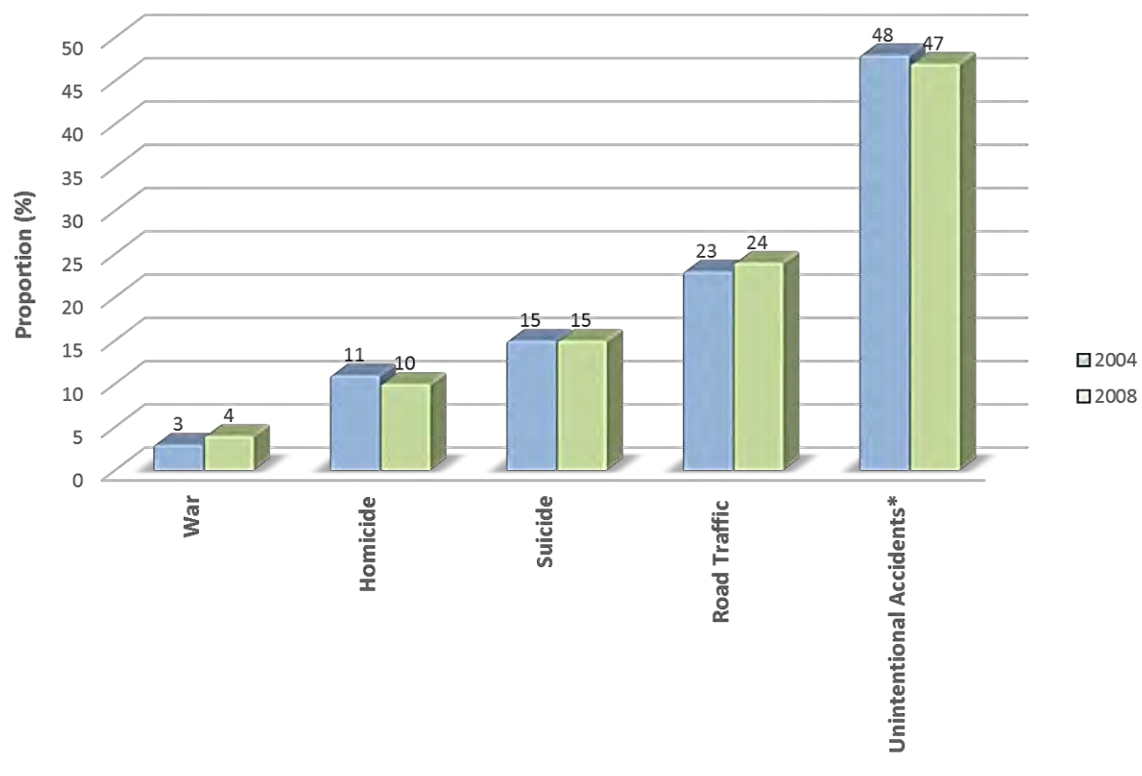
This review investigates and discusses the current realm of violence-related mortality, drug-dependence epidemiology, and the post-mortem drug toxicology of violence-related fatalities in both the international sphere and the Cape Town Metropolitan District in the Western Cape Province of South Africa. Reducing the magnitude of these major health burdens is important for the City of Cape Town due to the high rate of violence, and drug use and abuse [3–5]. International studies on toxicology of violent deaths report the presence of substances (alcohol and illicit drugs) in victims of homicides and suicides [6–9]. A connection between drug use and violence has been identified in the literature and appears to be related to the circumstances of drug use and the psychopharmacological effects of the drug taken on the substance user [10].

In an attempt to further understand the role of drugs of abuse in violent fatalities, the current review provides essential background on the different types of violence-related mortality and the drugs reported to be associated with them. Additionally, the authors comment on the potential of post-mortem toxicological analysis as an important tool to assess and understand the dynamics between drug use and violent fatalities. Finally, the review provides the rationale behind the need for, and importance of, post-mortem toxicological research in South Africa.

II. Global Violence, Injury and Death

According to the World Health Organization (WHO), violence is defined as the intentional and physical exertion of power or force to hurt or kill someone (including oneself) [11]. Acts of violence are among the leading causes of injury or physical damage, whether they are self-directed, interpersonal, and/or collective [12,13]. Injuries sustained through violence pose a great public health challenge worldwide. Violence-related fatal injuries are responsible for the deaths of approximately 5.8 million individuals worldwide annually, accounting for about 10% of global mortality. [12]. These violence- and injury-related fatalities are classified primarily as unnatural deaths (deaths not due to natural causes) and then sub-divided into four broad categories of manner of death as follows: homicides, suicides, accidents, and unclassified or undetermined deaths.

Homicides are defined as fatal injuries inflicted by another person to injure or kill someone, whether by omission or commission. Death by suicide results from the act of deliberately and intentionally killing oneself. Accidental deaths are deaths that occur through an accidental act, such as drowning, falling, and burning amongst others. The worldwide distribution of injury-related fatalities varies in terms of manner of death, cause of death, and mechanism of injury. Figure 1 illustrates the global distribution of reported injury deaths in 2004 [12,14–16]. Four years later, similar proportions were reported for the main manners of death (Fig.1) suggesting little improvements in the battle against fatal violence worldwide.



*Unintentional Accidents include poisoning, falls, drowning, fires, asphyxia, choking, animal and venomous bites, hypothermia and hyperthermia, as well as natural disasters.

Fig. 1 Distribution of reported injury deaths worldwide in 2004 and 2008 [12,14–16].

III. Fatalities in the Cape Town Metropole, South Africa

The Cape Town Metropolitan municipality of the City of Cape Town, the provincial capital of the Western Cape Province, is the second and tenth-most populated city in South Africa and Africa, respectively, with an estimated population of 3.87 million in 2014 [3]. The Metropole is divided into eight sub-districts: Western, Eastern, Khayelitsha, Klipfontein, Mitchells Plain, Northern, Southern, and Tygerberg (Figure 2) [17]. South Africa is a multi-racial nation and its inhabitants fall into one of the following racial groups as defined by the South African National Census of 2011 [18]: Black, White, Coloured, Indian or Asian and Other. Approximately 42.4% of Cape Town's citizens are members of the Coloured ethnic group, 38.6% are Black African, 15.7% are White, 1.4% belong are Asiatic, and 1.9% are considered as Other [19]. The Salt River mortuary, situated in Salt River in the Cape Town City Bowl Suburb, is a medico-legal facility that serves the West Metropole of the City of Cape Town, including the Southern Suburbs, Western Suburbs, Mitchells Plain, and Klipfontein sub-districts (Fig.2). The mortuary manages an average case load of 3500 autopsies per year [20].

Fig.2 Map of the Cape Town Metropolitan Health Districts. The dotted lines represent the metro health sub-districts within the West Metropole of Cape Town city [3,20].

The definitions of cause and manner of death may be used interchangeably by clinicians, forensic professionals, epidemiologists, nosologists, and laymen, but they are not synonymous. In South Africa, the Inquest Act (Act 58 of 1959) provides for the holding of inquests in unnatural deaths, which are cases of deaths occurring from other than natural causes [21]. Under this Act, and in accordance with the regulations of the National Health Act (Act 61 of 2003) [22], the body of a person who has allegedly died from other than natural causes shall be examined by the medical practitioner who performs a post-mortem examination of the body to assist the court in medico-legal investigations. The forensic pathologists of the Forensic Pathology Services (FPS) are responsible for determining the cause of death in these unnatural deaths in South Africa. All unnatural deaths undergo a standardised forensic autopsy, in which biological specimens may be sampled for toxicological, histological and other ancillary analyses. The cause of death is subsequently determined by the pathologists by means of analysing all evidence pertaining to the circumstances of death and the decedent's history, the autopsy findings, and the results of requested additional analyses.

In South Africa, the National Forensic Chemistry Laboratory (FCL) performs toxicological analyses for all medico-legal cases including driving under the influence of drugs and/or alcohol (DUI) and post-mortem investigations. At present, however, while toxicological testing of biological samples in all unnatural deaths is suggested within the national protocol, it is not currently feasible due to the large number of cases received by South African mortuaries as well as financial, staffing, and other challenges currently experienced by the laboratory, mortuary, and police. At the Salt River mortuary, blood is collected for alcohol concentration analysis in most cases. Samples for other toxicological analyses, however, are usually only collected for cause of death determination at the discretion of the forensic pathologist based primarily on the circumstances of death, the often limited history of the decedent, and the post-mortem findings at autopsy. The manner of death of an unnatural death is determined by the magistrate following presentation of all evidence from multiple agencies to assist in making that informed decision.

IV. Prevalence of Violence-related Fatalities in South Africa

IV.A Homicides

According to the latest United Nations Office on Drugs and Crime (UNODC) report, the reported deaths of approximately half a million individuals worldwide in 2012 were homicidal, with approximately 36% occurring in the Americas, 31% in Africa, 28% in Asia, 5% in Europe, and 0.3% in Oceania [23]. In the same year, Southern Africa, which includes Swaziland, South Africa, Namibia, Lesotho, and Botswana, had a homicide rate over four times higher than the current global average homicidal rate of 6.2 per 100,000 individuals [23]. Within all the countries that record crime statistics, South Africa's murder rates are among the highest [24]. Additionally, the number of reported homicide cases recorded and reported by the South African Police Services (SAPS) have seen considerable changes over the past 10 years [25]. As illustrated in Figure 3, from 2004/5 to 2013/14, the recorded homicide cases in South Africa decreased by 9.2% but increased by 1.4% during the past 5 years (2009/10-2013/14) [25]. More recently, during the past fiscal year (2013/14), the reported homicide figures increased by 5%. With a homicide rate of 32.2 per 100,000 in 2013/14, South Africa's rate is more than five times higher than the global average (6.2 per 100,000) homicide rate recorded in 2013 [23]. There may have been changes in the population counts that resulted in changing percentages of homicide rates. In addition, these figures are only indicative of cases that have been reported to the SAPS, in which some cases may be preliminary homicide and determined as accidents at a later stage. Despite these limitations, there is a clear indication that South Africa has a very high rate of homicide.

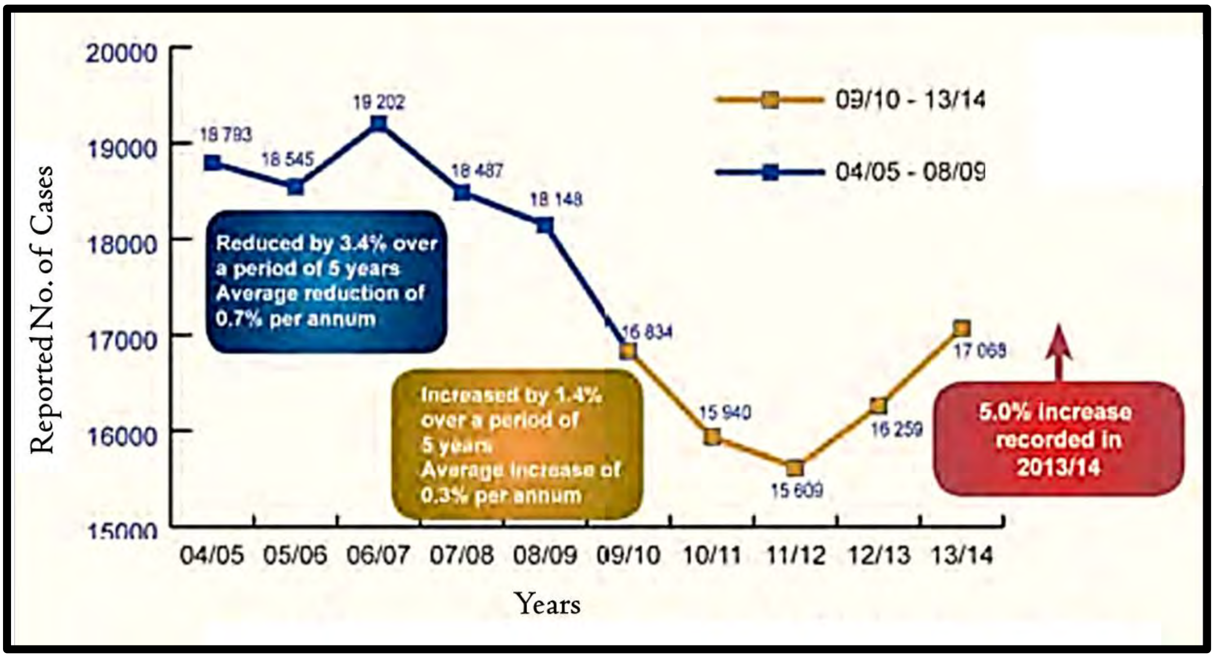


Fig. 3 Reported homicide cases in South Africa from April-March 2004/5-2013/14 [25].

IV.B Suicides

With a mortality rate of 11.4 per 100,000 individuals, death distinguished as suicides accounted for up to 1.4% of total mortality worldwide in 2012 [26]. Based on current trends, it is estimated that suicide will constitute up to 2.4% of the global burden of disease by the year 2020

[27,28]. Depending on research methodologies and sampling processes, the annual South African suicide rate ranges from 11.5 per 100,000 to 25 per 100,000 individuals [29]. However, recent literature suggests that all forms of suicidal behaviour among all socio-demographic population groups in South Africa are on a continuous but slow rise [30]. Studies on mortality surveillance estimated that suicide represented 8.9%, 10%, and 9.9% of total injury-related deaths nationwide in the years 1999, 2000, 2001 [31]. Over the 7-year period (2002-2008), on average, over 9.6% of all unnatural fatalities were reported as suicide [32]. In a more recent study, the suicide mortality rate of South Africa was reported to be 13.4 per 100,000 individuals in 2009, which is higher than the 2012 global suicide mortality rate of 11.4 per 100,000 [33]. Once more, the South African reported rates rely heavily on accurate reporting data and determination of manner of death, but there is an indication that the country has a high suicide rate (although lower than homicide rate).

IV.C Accidental Deaths

Although death by accidental injury constitutes a health burden worldwide, unlike the other types of violent death, research carried out on unintentional and accidental deaths (apart from road traffic accidents) is not as extensive. Given the frequency of road traffic accident fatalities, most literature reports categorise this as a separate category of unnatural death. The leading causes of accidental death differs across countries and within different regions of the same country [34,35]. In South Africa, the two leading causes of accidental deaths apart from transport-related accidents are fire-related and drowning fatalities [33,36]. In 2001, accidental fatalities accounted for over 3.5 million deaths worldwide at a rate of 61 per 100,000 individuals annually, which represented 66% of all injury fatalities [37]. This rate includes transportation deaths which accounted for 34% of the accidental fatalities. The accidental mortality rate increased to over 3.9 million deaths in 2004 (33% due to road traffic injuries) with more than 90% of these occurring in low- and middle-income countries, including South Africa [14,35]. As depicted in Figure 1, accidental deaths (including drowning, fires, falls, and other causes) comprise the largest proportion of violence- and injury-related deaths worldwide [12,15].

V. Drug Use and Abuse

Historically, any substance or compound with extraordinary effects on human behaviour was commonly called a ‘drug’ [38]. Around the world, the use, meaning and nature of drugs differs between communities and countries. Today, a drug refers to any chemical substance or compound, natural or synthetic that when administered causes physiological, biochemical, and psychological changes in the human body [39]. Drugs commonly abused include licit substances such as alcohol, caffeine and tobacco, the use of which have become socially acceptable, and illicit substances such as cannabis, amphetamines, and cocaine, which are controlled or scheduled in most countries [39]. Drugs can be classified in different ways according to their chemistry, indications, mode of action, and effects on the body. A psychoactive or psychotropic drug is a substance, used for medical or recreational purposes, that primarily acts on the central nervous system to alter mental processes including perception, consciousness, behaviour, mood, and thoughts [39]. The terms psychotropic or psychoactive drugs are the most descriptive and neutral for the whole class of substances (licit and illicit) of interest to drug policy [40]. Substance use and abuse are a major public health issue worldwide. The WHO 2002 report found that almost 9% of the total burden of disease resulted from the use of psychoactive substances [41].

Drugs of abuse can be broadly divided into seven different class of drugs: (i) stimulants – increase brain activity (e.g. amphetamines, cocaine, caffeine, nicotine, and methylphenidate), (ii) depressants – decrease brain function (e.g. methaqualone, barbiturates, alcohol, and benzodiazepines), (iii) hallucinogens – alter feelings and perception (e.g. ketamine, phencyclidine, and lysergic acid diethylamide), (iv) cannabis – has effects of depressants, stimulants and hallucinogens (e.g. marijuana, hashish), (v) narcotic analgesics (opioids/opiates) – sedating painkillers (e.g. oxycodone, codeine, morphine, heroin, opium), (vi) inhalants – volatile substances with mind-altering effects (e.g. gasoline, propane, glue) and (vii) other drugs of abuse such as steroids, dextromethorphan, muscle-relaxants, anti-psychotics, and anti-depressants [42]. Substance use refers to the recreational or occasional consumption of alcohol or other drugs, while substance abuse is defined as the recurrent, hazardous and maladaptive use of drugs (licit and illicit) with negative consequences, such as increased risk of life-related complications (e.g. mental, physical, socio-economical, and health issues) and the inability to control drug consumption [43,44]. Substance dependency on the other hand, refers to the complete addiction to a substances when the individual experiences withdrawal symptoms and starts developing a tolerance for the drug [44]. Drugs of abuse tend to have addictive properties that result in their abuse.

V.A Worldwide

The global prevalence of reported illicit drug use and abuse remains stable in the past three years, with approximately 5.2% of the global population (246 million individuals), between 15 and 64 years of age, reported to have used an illegal substance in 2013 (Fig.4B) [45]. Similarly, the global extent of reported problem drug use – including individuals suffering from illicit drug-use disorders and drug-dependence – appears to remain unchanged over the 3-year period, accounting for 0.6% of the world's population between 15-64 years of age (27 million individuals) [45]. According to the 2015 UNODC report, the most widely used illicit substance worldwide is cannabis (marijuana), followed by opioids (such as heroin), opiates (substances derived from the opium poppy plant, such as morphine, codeine), cocaine, and amphetamine-type stimulants (ATS), which are divided into amphetamines (amphetamines and methamphetamines), tryptamines, and phenethylamines (e.g. MDMA – shortened term for 3,4-methylenedioxy-methamphetamine) (Fig.5) [45]. It is important to mention that the above reported figures only provides an indication of reported drug use due to limitation in reporting accuracy such as disregarding changes in population counts. Moreover, a number of novel (or new) psychoactive substances (NPS) such as synthetic cannabinoids and cathinones, the use of which are not accurately reported, have entered the drug market and replaced traditional drug use. Therefore, the drug market worldwide is ever-changing, but it is clear that drugs are being used within our society, and if a particular drug declines in use, its place is often replaced with another.

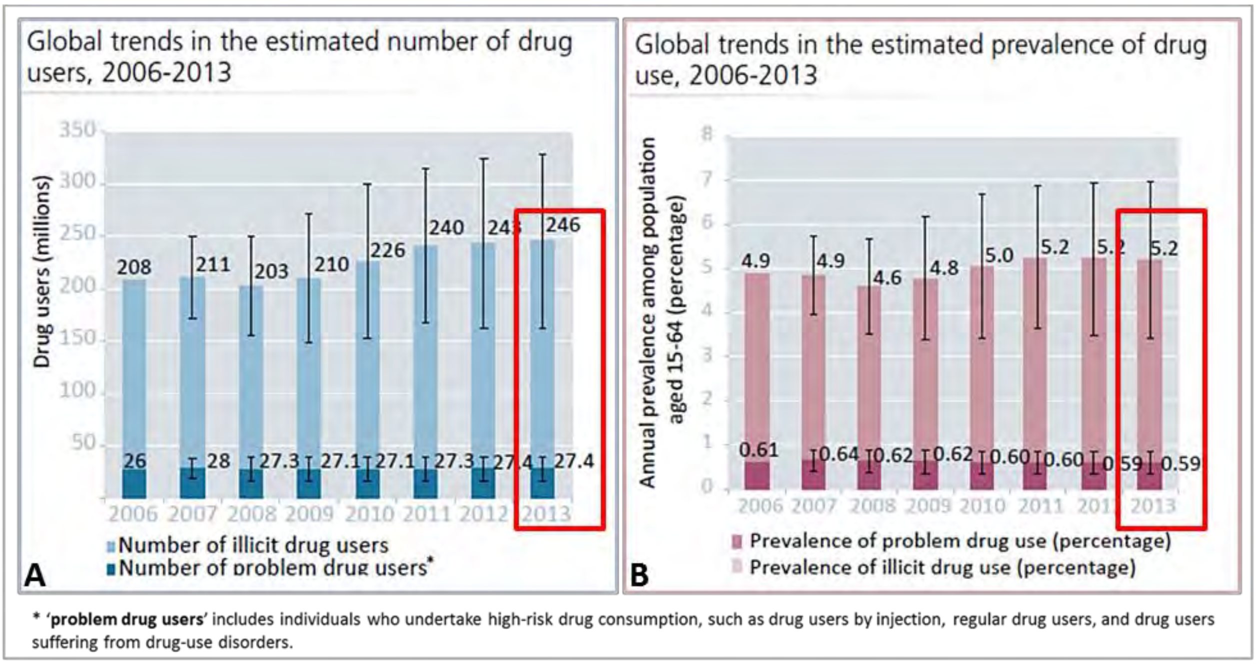


Fig.4 Global trends in the estimated (A) number of drug users and (B) prevalence of drug use, 2006-2013 [45].

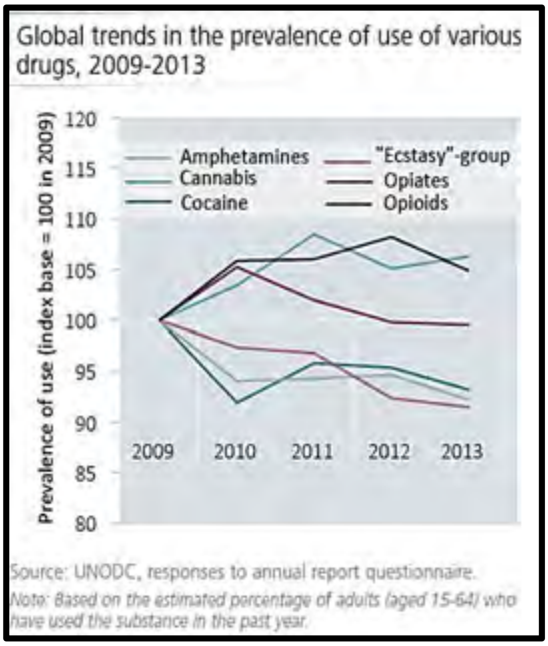


Fig.5 Global trends in the prevalence of use of various drugs, 2009-2013 [45].

V.B South Africa

Historically, information on substance use and abuse in South Africa has been scarce. During the post-apartheid period of the late 1990's, drug-related data was generated from school surveys, post-mortem reports, police arrests and drug seizures, as well as national survey-type studies [46]. Today, there exist various public-health surveillance and monitoring systems of substance use and abuse, such as the South African Community Epidemiology Network on Drug Use (SACENDU), which provides more reliable information on the substance abuse trends across the country [47]. However, despite the successful implementation of these systems, there remains a paucity of complete and systematic data to fully represent the whole South African population [48]. For instance, the SACENDU figures only involve individuals who managed to get access to the available treatment facilities and hence the data is unlikely to be fully representative of the general population.

According to UNODC's best estimates of annual prevalence of reported illicit drug use in the adult population (aged 15-64 years) in South Africa, cannabis is the most widely used illicit substance, followed by ATS, cocaine, ecstasy-type group, and opiates (including opioids) [49]. Within the country, the City of Cape Town continues to battle the increasing use of crystal methamphetamine (locally known as 'tik'), which is the primary drug of abuse (Fig.6), followed by cannabis (or 'dagga'), heroin, methcathinone (or 'Kat'), methaqualone (or 'Mandrax'), crack cocaine, and OTC/PRE (over-the counter and prescription drugs) (Fig.7) [50].

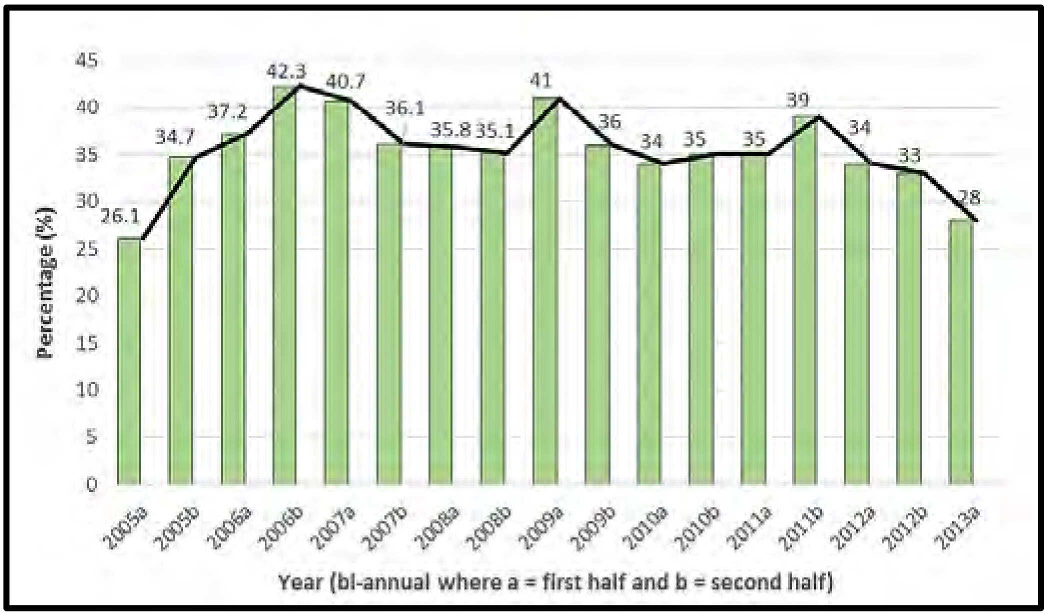


Fig.6 Trends in treatment demand (from 26 specialist treatment centres) for methamphetamine as the primary substance in Cape Town, South Africa, 2005-2013 (biannual data) [50–52].

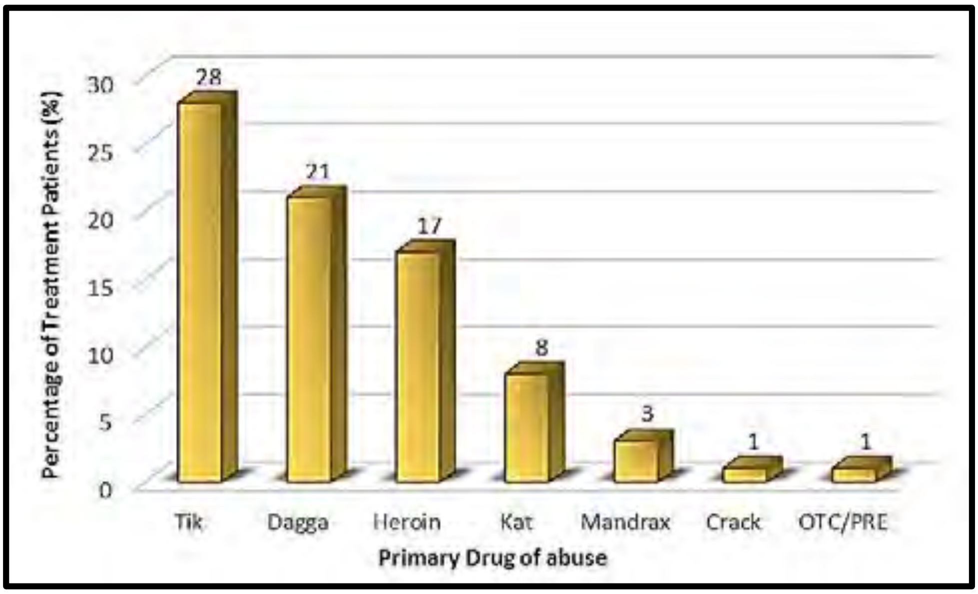


Fig.7 Trends in treatment demand (from 26 specialist treatment centres) for the primary substance in Cape Town, South Africa, 2013 Jan-Jun [50].

VI. Substance Use and Violence

The literature has identified an association between psychoactive substance use, primarily alcohol, and violent behaviour, which, to date, has not been fully characterized due to the complexity of its aetiology [53–55]. Violence is reported to occur during acts of drug-seeking, chronic and acute intoxication, drug-induced psychotic paranoia, drug withdrawal, and relapse [53]. Most of the common drugs of abuse have the potential to trigger violent behaviour, however, they do so through different mechanisms depending on the drug type, quantity and administrative route used, and the individual's drug use pattern [56]. The reported incidence of alcohol-induced aggression and violent behaviour is high due to its licit nature and well characterised psychopharmacological effects, such as central nervous system (CNS) depression, disinhibited behaviour, motor skills impairment, and antisocial impulses [53,57]. The use of psychostimulants, such as amphetamines and cocaine, have also been suggested to play a contributory role in violent behaviour due to their psychopharmacodynamic properties producing aggression, paranoia, delirium, and psychosis [53,58,59]. For instance, Darke *et al.* (2010) found that the use of the methamphetamine is associated with an increased risk of violent offending [60]. Boles and Miotto (2003) stated that the use of amphetamines is related to pharmacological and systemic violence, however, there is no conclusive evidence on the causal nature of the relationship [53]. Additionally, cocaine has been shown to be associated with the perpetration of violent crimes worldwide, but particularly in the United States where it is one of the most commonly abused illicit substances [53,61–63]. On the other hand, the use of other substances such as cannabis and opiates have been found to reduce aggressive behaviour and violent actions temporarily promoting sedation, CNS depression, relaxation, and mood alterations, which are limited in their association with aggressive and violent behaviours [53,54,64]. At present, the association between substance use and violent events requires further investigation to greater understand the specific role and characteristics – whether causal, contributory, precipitating or predisposing – that the use and abuse of drugs plays in the risk of violent perpetration and victimisation [53,64,65].

According to Goldstein's (1985) conceptual framework, the mechanisms behind the suggested link between substance use and violence relate to: (a) the direct psychopharmacological effects of the substance on human behaviour (e.g. intoxication, disinhibiting drug effects, symptoms of withdrawal, paranoia, agitated delirium, and more); (b) the economic-compulsive dimension of the situation, which refers to the use of violence to obtain money or other forms of currency for personal drug use (e.g. dependent use, robbery); and (c) the systemic violence, which refers to violent events intrinsic to the lifestyle activities, and business methods of drug traffickers and distributors (e.g. violence between dealers), all of which may substantially increase the risk of violence [58,10]. The application of Goldstein's tripartite model of the drug-violence relationship to the South African context is challenging, particularly when trying to characterise the role that the direct pharmacological effects of drugs of abuse plays in violent behaviour [66]. Other countries, such as the United States, possess numerous scientific studies that support the relevance and significance of the tripartite conceptual framework in understanding illicit drug-related violence [10,66–68].

On the contrary, in South Africa, research on the nature of association between violence and substance use is limited [66,69]. There is a general focus on the 'epidemiology' of health burdens, such as injury mortality, violence, and drug use, at the regional (e.g. provinces, metropolitan areas), community (e.g. ethnic population groups, different age groups) or organizational levels (e.g. SACENDU, SAPS) in South Africa [24,25,47,69–72]. Furthermore, although South Africa possesses surveillance and monitoring systems for fatal injury mortality and substance use in treatment centres such as the National Injury Mortality Surveillance System (NIMSS) and SACENDU, respectively, the self-reported aspect of epidemiological investigations presents a limiting factor in terms of accuracy and reliability, and reports specific to the incidence of violence associated with illicit drug use remains scarce. In addition, from a toxicological standpoint, analysts require adequate toxicological specimens, which are not routinely collected in South Africa, for the identification and quantitation of drug concentrations which allow toxicologists to provide interpretations concerning the individual's state of intoxication. The common under-reporting of violent behaviour in South Africa presents another limitation.

Due to inaccuracies and/or the lack of circumstantial evidence (e.g. eye witness statements), fear of victimisation, ignorance of the laws, no access to the police, and corruption, many acts of violence are disregarded across the country [73]. With self-reported drug use, it is difficult to distinguish the nature of the drug taken (due to possible additives and adulterants), to obtain reliable information on the history of drug use, and comment on the state of intoxication [74]. As a result of all these constraints, information associated with drug use and violence in South Africa is limited in its scientific value. Using comprehensive and coherent conceptual frameworks such as the Goldstein model as well as toxicological tools may be of significant assistance in further research and investigation into the possible association and nature of drug use and violence in South Africa.

VII. Substance Use and Violent Fatalities

It is reported that the use of psychoactive substances increases one's risk of becoming a victim or perpetrator of violence [23]. Studies on substance abuse-related violence indicate that substance use and dependence may increase the user's risk of morbidity and mortality due to injury and increased violent behaviour [53,54,71]. Additionally, the mortality rates among drug users are higher than those of the general population, and deaths due to unnatural causes are more prominent in drug-dependent individuals than those due to natural causes [1]. The most common reported causes of death within dependent substance users are disease and drug overdose, however, some fatalities related to substance use and abuse are a result of violence, in the manner of homicide, suicide, and accidental [1].

VIII. Toxicology of Violent Deaths

As mentioned previously, toxicological analyses are routinely requested by forensic pathologists, coroners, or medical examiners in most, if not all, cases of unnatural death in most developed countries. However, service-obtained post-mortem toxicological data from victims of violent death and investigation into the role of these drugs in these fatalities are not routinely published in research literature internationally [62,75].

VIII.A Toxicology of Homicide Victims

Currently, only a handful of studies have investigated drug levels in homicidal victims around the world [6–8,76–82]. According to Darke's (2010) comprehensive review on the toxicology of homicides; alcohol and/or illicit substances are present in half or more of the victims, with alcohol being the most commonly detected, followed by three major drug classes: psychostimulants (such as amphetamines and cocaine), cannabis, and opioids [83]. Most of these homicide studies (18 out of 31 studies identified by the authors) were conducted in the United States with the study methodology either being based upon prospective post-mortem toxicological analyses, retrospective review of toxicological data collected from mortuary records, or a combination of both [71,76–79,81–96] (Table 1). The review reveals that cocaine is the most commonly detected illicit substance in the cohort of United States homicide victims studied, and its use appears to be associated with increased risk of death from firearm-related violence [7,76,77,83].

The remaining 13 toxicology studies among homicide victims were conducted in other countries, including Denmark (1), Sweden (2), Australia (3), Ireland (2), South Africa (2), Finland (2), and United Kingdom (1) [7,83,97–108] (Table 1). The toxicological findings among homicide victims in different countries reveal that the types of drugs detected differed between studies but the general consensus is that substances (licit and illicit) are prevalent in homicidal deaths, which appears to be a prevalent manner of death among drug users. Altogether, the authors recognise the need to widen the scope of research in toxicology in homicide cases to include those in more under-developed countries, which would allow for wider comparisons. They also indicate the importance of using homicide toxicology data to further probe the assessment of drug intoxication and incidence of violent homicide.

Table 1 Toxicology of homicide victims in comprehensive studies as listed by Darke et *al. 2010* [83].

Study	Country	Findings
Alcohol as a factor precipitating aggression and conflict behavior leading to homicide. Virkkunen (1974) [106].	Finland	Alcohol 68%, mean BAC* > 0.20g/100mL
Drugs and homicide in Erie County, New York. Abel (1987) [85].	USA	Alcohol 45% Drugs 4%
Homicide in Cape Town, South Africa. Duflou *et al.* (1988) [104].	South Africa	Alcohol 63%, 30% BAC* > 0.20 g/100mL
Cocaine and homicide in Memphis and Shelby County: an epidemic of violence. Harruff *et al.* (1988) [89].	USA	Drugs: cocaine 17%
Injuries due to deliberate violence in areas of Denmark. IV. Alcohol intoxication in victims of violence. The Copenhagen Study Group. Albrektsen *et al.*(1989) [97].	Denmark	Alcohol 42% (assault survivors)
Assaults in south east London. Hocking (1989) [107].	UK	Alcohol 50% (assault survivors)
Crack and homicide in New York City, 1988: a conceptually based analysis. Goldstein *et al.* (1989) [88].	USA	Drug dealers 34%
Homicide: drinking by the victim. Welte & Abel (1989) [96].	USA	Alcohol 42% (71% of positives BAC* > 0.10g/100mL)
Cocaine in Wayne County Medical Examiner's cases. Hood *et al.* (1990) [90].	USA	Drugs: cocaine 27%
Homicide committed by abusers of alcohol and illicit drugs. Lindqvist (1991) [99].	Sweden	'Intoxicated' 86%
Women, homicide and alcohol in Cape Town, South Africa. Lerer (1992) [103].	South Africa	Alcohol 62%, 56% BAC* > 0.10 g/100mL
Drug use among homicide victims. Changing patterns. Garriot *et al.* (1993) [82].	USA	Substances 69% Alcohol 57% Drugs: cocaine 11%, heroin 4%
Gang-related homicides in Los Angeles County. Rogers (1993) [92].	USA	Gang homicide: alcohol 52%, cocaine 20% Non-gang homicide: alcohol 48%, alcohol 28%, opioids 4%
Homicide in Ireland 1972–1991. Dooley (1995) [101].	Ireland	Alcohol 42%
The quantification of drug-caused morbidity and mortality in Australia 1995. English *et al.* (1995) [109].	Australia	Aetiological fraction alcohol = 0.43
Cocaine, opiates and ethanol in homicides in New York City: 1990 and 1991. Tardiff *et al.* (1995) [79]	USA	Alcohol 35% Drugs: cocaine 31%, heroin 12%
Alcohol and illicit drug abuse and the risk of violent death in the home. Rivara *et al.* (1997) [71].	USA	Case control: Higher levels use of alcohol and illicit drugs among cases.
Victims of criminal homicide in Sweden: a matched case-control study of health and social risk factors among all 1739 cases during 1978–1994. Allgulander & Nilsson (2000) [98].	Sweden	Alcohol dependence 9% Drug dependence 1%

The relationship between substance use, drug selling, and lethal violence in 25 juvenile murderers. McLaughlin *et al.* (2000) [91].	USA	Substances 27% Alcohol 18%
Homicide in Ireland 1992–1996. Dooley (2001) [102].	Ireland	'Intoxicated' by alcohol and/or drugs 42%
The role of alcohol in accident and violent deaths in Finland. Lunetta *et al.* (2001) [105].	Finland	Alcohol 63%
The role of alcohol use in intimate partner femicide. Sharps *et al.* (2001) [93].	USA	Substances 21% Alcohol 18% Drugs 3% Alcohol + drugs 4%
An empirical analysis of deviant homicides in Chicago. Varano & Cancino (2001) [95].	USA	'Deviant homicide': alcohol 45% 'Non-deviant homicide': alcohol 55%
Drugs and firearms deaths in New York City, 1990–1998. Galea *et al.* (2002) [76].	USA	Substances 55% Alcohol 27% Drugs: cocaine 24%, cannabis 20%, opioids 10%
Sharp injury fatalities in New York City. Gill & Catanese (2002) [87].	USA	Substances 61%
Gunshot fatalities in children and adolescents in New York City. Gill *et al.* (2003) [78].	USA	Substances 56% Alcohol 14% Illicit drugs 48% Drugs: cannabis 40%, cocaine 4%
Exploring the drugs-homicide connection. Varano & Cancino (2004) [94].	USA	Drug involvement of either offender or victim 49%
Drug and alcohol use as determinants of New York City homicide trends from 1990 to 1998. Tardiff *et al.* (2005) [77].	USA	Substances 59% Alcohol 30% Drugs: cocaine 28%, cannabis 18%, opioids 11%
Homicide in Australia: 2005–2006 National Homicide Monitoring Program annual report. Davies & Mouzos (2007) [100].	Australia	Substances 49% Alcohol 36% Illicit/prescription drugs 24% Alcohol + illicit/prescription drugs 9%
Homicides committed by youth assailants: a retrospective study. Adeagbo *et al.* (2008) [86].	USA	Substances 74% Alcohol 11% Drugs: cocaine 20%, cannabis 14%, diazepam 5%
Toxicology and circumstances of death of homicide victims in New South Wales, Australia 1996– 2005. Darke *et al.* (2008) [7].	Australia	Substances 63% Illicit substances 33% Multiple substances 25% Alcohol 42% Drugs: cannabis 21%, opioids 11%, psychostimulants 10%.

[*] BAC = Blood Alcohol Concentration, measured in g/100mL

Table 2 Post-mortem toxicology of homicide victims in South Africa.

Year	Study Title	Author(s)	Objectives	Population	Findings + Drawbacks
1964	Violent Deaths and Alcoholic Intoxication	Le Roux & Smith [111]	• To determine whether there exists any correlation between the incidence of alcohol intoxication and the frequency of violent deaths.	All medico-legal autopsies performed in the Cape Peninsula for the year 1962.	At least 64% of all homicidal victims has a positive blood alcohol levels. The post-mortem concentration may not speak to intoxication at time of event due to individual factors and post-mortem artefacts.
1984	Homicidal penetrating incised wounds of the thorax	Moar [112]	• An autopsy study of 52 cases.	Medico-legal autopsies of victims of homicidal assaults with weapon at the Johannesburg and Diepkloof government mortuaries in 1981.	Almost 80% of the victims had a positive blood alcohol level ranging from 0.01 to 0.34 g/100mL; 58% BAC* between 0.15-0.29 g/100mL indicative of a significant degree of intoxication. There was no indication of a control group.
1988	Homicide in Cape Town, South Africa	Duflou *et al.* [104]	• To perform a retrospective analysis of homicide victims. • To establish the pattern of homicide in Cape Town.	All autopsies done on homicide victims at the police mortuary in Salt River, Cape town, between Jan-Jun 1986.	Alcohol was detected in 63% of cases; 8.4% BAC* > 0.30 g/100mL, indicating gross alcohol intoxication.
1992	Women, Homicide and Alcohol in Cape Town, South Africa	Lerer [103]	• To describe female homicide in Cape Town, its relationship with sex, race, age, female suicide and blood alcohol concentration	Female homicides admitted to the Salt River Mortuary between Jan 1990 and Jul 1991	56% of all female homicides studied tested positive for BAC* > 0.10g/100mL.
2009	Alcohol use and its role in female homicides in the Western Cape, South Africa	Mathews *et al.* [113]	• To describe the patterns if blood alcohol concentration (BAC) at the time of death for female homicide victims • To explore the factors associated with having an elevated BAC	A subsample of a national, representative retrospective mortuary-based study of female homicides ages 14 years and older in the Western Cape in 1999	A positive BAC* was detected in 62% of victims with mean BAC* 0.20 g/100mL. 44.4% BAC ≥ 0.15 g/100mL which was categorized by the authors as "very drunk". Authors do not account for individual factors such as tolerance, post-mortem artefacts, and post-mortem interval. These concentrations may not reflect those at "time of death" as they state.

* BAC = Blood Alcohol Concentration, measured in g/100mL

Unfortunately, forensic research involving toxicological analysis of samples from homicide victims has received little attention in the South African society, especially given the high rates of recorded homicidal deaths throughout the years [23,24,104,113,114]. In fact, to the author's knowledge, only five studies have been conducted in South Africa that examine the presence of alcohol in homicidal cases, while no studies report toxicological data in victims of homicide [103,104,110–112]. As illustrated in Table 2, the studies were primarily limited to the Western Cape area, based on outdated population statistics and data collected from mortuary reports, and only blood alcohol analyses were performed. Drawbacks specific to each study are also mentioned in Table 2. This calls for attention to the need for new research focusing on post--mortem toxicology of homicide victims, including both drugs and alcohol analyses. These older studies report the detection of alcohol above 0.00 g/100mL as a 'positive BAC'. Interestingly, consistent with recent international forensic research, alcohol was detected in half or more of the cases investigated in each of these five studies. However, one should bear in mind that these results should be interpreted in the light of the extensive and complex nature of post-mortem toxicological interpretation. To the best of the authors' knowledge, the presence of substances other than alcohol in post-mortem homicide fatalities has not been investigated in a South African setting to date. Given the international findings of drug use in homicide victims and the increased prevalence of both homicide and drug use in South Africa, further investigation into post-mortem toxicology in homicide cases is warranted to provide information that may contribute to further understanding the patterns of homicide-related violence associated with drug use and abuse.

VIII.B Toxicology of Suicide Victims

There is evidence of an association between substance use, suicidal attempts, and completed suicide in the literature [115–119]. Systematic comparative studies have shown that drug-dependent individuals exhibit higher levels of suicide risk factors than the general non-substance dependent individuals [1,75,119–121]. For example, studies report the relatively higher risk of completed suicide by substance users differing across the detection of a variety of drugs: benzodiazepines (45 times), opioids (14 times), alcohol (6 times), and cannabis (4 times) [119,121]. Due to the complex and multifaceted relationship between the use of drugs and suicide, some researchers proposed that toxicological monitoring of suicide deaths may assist in unravelling the contribution of substances in the occurrence of these violent deaths [75]. According to the literature, systematic and comprehensive toxicological research is not sufficiently carried out worldwide probably due to limited resources, non-comprehensive testing, and results only published in crime reports [122].

In this review, the author describes four studies that comprehensively examined the prevalence of psychoactive substances in victims of suicides without limiting the studies to a specific population group, age group, drug type or method of suicide, as shown in Table 3 [75,122–124]. Consistent with suicide risk factors, these comprehensive studies report high levels of drug detection amongst suicide victims, with alcohol being the most commonly detected substance, followed by pharmaceuticals such as benzodiazepines and antidepressants. Through the years, a number of studies have evaluated the relationship between these psychotropic substances and the risk of suicide. Benzodiazepines are pharmaceutical drugs prescribed for anxiety, insomnia, and muscle relaxation. With anxiolytic and amnesic properties, the pharmacological side effects of benzodiazepines include sedation, slurred speech, loss of inhibition, and memory impairment. Individuals may consume these to ease the process of committing suicide. Antidepressants medication has been shown to potentially precipitate suicidal behaviour [125,126]. For instance, several studies indicated that antidepressants such as fluoxetine may increase symptoms of depression and encourage suicidal thoughts in some individuals [23-24]. Many individuals who suffer from depression may commit suicide with these medications in their body from therapeutic or toxic ingestion.

Other studies on suicide toxicology focus on a specific substance of abuse [61,63], drug class [127], and/or on a particular method of suicide [128]. In each of them, drugs of abuse were detected in half or more of the cases, with alcohol and pharmaceuticals as the main detected substances [129]. The findings indicate that substances are commonly found in suicide victims, and emphasize the need to monitor toxicology in suicide cases to identify patterns of substance use. The association between drug use and suicide fatalities includes data obtained across a range of methods of committing suicide [6,9,129]. In cases of drug overdose or poisoning, positive toxicological data are expected. In contrast, the possible association between suicides due to non-substance toxicity and drug use is more complex and the aetiology remains unclear. An important study, conducted in Australia by Darke *et al.* (2009), specifically investigated the toxicology and circumstances of non-overdose suicide fatalities in an attempt to distinguish the association of drugs with violence-related suicide [9]. Consistent with previous studies, alcohol was the most frequently detected substance, followed by pharmaceuticals such as antidepressants, antipsychotics, and benzodiazepines (Table 4) [9]. The most common detected illicit drug was cannabis, followed by opioids (e.g. morphine) and psychostimulants like methamphetamine [9]. The findings are consistent with other reports stating that substance users most commonly commit suicide by means other than drug overdose such as hanging, jump, gunshot, sharp and blunt force trauma [1].

Table 3 Comprehensive studies on the toxicology of suicides investigating the presence of drugs in victims.

Study	Objectives	Population	Findings
Toxicologic findings in suicide: a 10-year retrospective review of Kentucky medical examiner cases. Shields *et al*. (2006) [122].	10-year (1993-2002) retrospective study on the toxicological findings of medical examiner cases of suicide in Kentucky. Present the prevalence of psychoactive substances detected at autopsy.	All suicide cases performed at the medical examiners' offices in Kentucky (*n*=2864).	▪ 67.5% of cases were a positive for at least one substance. ▪ 38.3% of cases has a positive BAC. 13.6% cases BAC < 0.1%, and 24.5% cases BAC ≥ 0.1%. ▪ Cannabis (15.6%), antidepressants (14.9%), and benzodiazepines (12.7%) are the most common drugs detected. ▪ The leading cause of suicidal death was firearm injury (67.4%), hanging (13.7%), and drug toxicity (also called overdose) (9.9%). ▪ Death by substance toxicity (overdose and poisoning) 10%, Death by other methods 90%.
Patterns of psychoactive substance detection from routine toxicology of suicides in Mobile, Alabama, between 1990 and 1998. Dhossche *et al*. (2001) [2].	Describe the toxicological data collected routinely in suicide victims of Mobile County during Oct 1990 - Sep 1998. Examine patterns in detection of specific types of psychoactive substances as well as the overlap of multiple substances.	96% (*n*=333) of the N=346 suicides occurring in Mobile County between Oct 1990 - Sept 1998.	▪ Substances were detected in 72.7% of cases; 93.8% of which were psychoactive substances and 6.2% were positive for medications not considered psychoactive. ▪ 33% of cases BAC* ≥ 0.05 g/100mL. ▪ The most frequent substances detected, other than alcohol, were antidepressants (19.8%), opiates (15%), and benzodiazepines (13.5%). ▪ Death by substance toxicity (overdose and poisoning) 12%, Death by other methods 88%
Alcohol and drugs in suicides. Ohberg et *al.* (1996) [123].	Assess alcohol and drug use nationwide Apr 1987 – Mar 1988. Toxicological screening of all consecutive suicides committed in the country in that 1-year period.	All suspected suicides (*n*=1348) in Finland during the 1-year study period.	▪ 41.6% of all suicides tested positive for drugs (positive toxicology). ▪ 35.9% of all suicides had a BAC* ≥ 0.05 g/100mL. ▪ The most frequently detected drugs were benzodiazepines (23.1%), neuroleptics (12.8%), and antidepressants (8.2%). ▪ The leading method of suicide was hanging (33.0%), followed by poisoning or overdose (28.5%), and firearms (20.4%). ▪ Death by substance toxicity (overdose and poisoning): 28.5%, Death by other methods: 71.5%.
Psychoactive substances in suicides. Comparison of toxicologic findings in two samples. Dhossche *et al*. (2001) [130].	Comparison of comprehensive toxicologic findings on psychoactive substances from consecutive suicides in San Diego, California, from 1981–1982, and Mobile, Alabama, between 1990 and 1998.	Compare toxicologic findings in n=179 suicides in San Diego County, California, between 1981 and 1982, and n=333 suicides in Mobile county, Alabama, between 1990 and 1998, were compared.	▪ 68% of suicides in San Diego were positive for positive toxicology. ▪ Alcohol was detected in about 30% of suicides in San Diego. ▪ The main drugs detected were cannabis (15.6%), opiates (11.7%), and benzodiazepines (9.5%). ▪ In San Diego, 21% suicide victims died of overdose and 79% died of other methods.

* BAC = Blood Alcohol Concentration, measured in g/100mL of blood.

Unfortunately, comprehensive toxicological data in suicide cases are not available in South Africa, as toxicological analysis is not routinely requested in non-overdose cases. Given the recent international research findings, monitoring and reporting of toxicological data in suicidal fatalities, including non-overdose cases, is required to further understand the role of psychoactive drug use in this category of violent death [9,128]. Additionally, considering the rates of reported suicidal death and the epidemiology of drug use in South Africa, there is a need to investigate the contribution of substance use and abuse in these violence-related fatalities.

Table 4 Toxicology of non-overdose suicide cases in New South Wales, Australia 1997-2006. Reproduced from Darke *et al.* 2009 [9].

Substances	All (N = 1415) %
Alcohol	**40.6**
Pharmaceuticals	**26.5**
Benzodiazepines	*15.1*
Antidepressants	*12.5*
Antipsychotics	*5.2*
Cannabis	**10.5**
THC acid	*8.3*
Δ-9-THC	*7.8*
Opioids	**9.3**
Morphine	*5.7*
Codeine	*5.5*
Methadone	*2.0*
Psychostimulants	**6.5**
Methamphetamine	*4.0*
Cocaine/Benzoylecgonine	*1.8*
MDMA (3,4-methylenedioxy-methamphetamine)	*1.4*

VIII.C Toxicology of Victims of Accidents

Accidental deaths, apart from road traffic fatalities, which do not fall into the scope of this review, may occur in various circumstances such as drowning, falling, burning, electrocutions, natural disasters, occupational-related or recreational activities, asphyxia, animal stings or bites, and other external causes (sharp and blunt force). Several international studies have examined the relationship between substance use and the risk of fire-related injury and deaths [131–133]. However, only a few of them have comprehensively investigated the role of substance use and abuse in victims dying from fire-related incidents. For instance, in New-Jersey, United States, Barillo and Goode (1996) found that about 30% and 15% of the tested fire-related deaths were positive for alcohol and other drugs of abuse, respectively, over a 7-year period [134]. The most commonly detected drugs were cocaine, benzodiazepines, barbiturates, and cannabis. The researchers suggest that one possible consequence of inebriation and drug intoxication may impair coordination and judgement abilities, hence increasing the risk of initiating a fire or being exposed to a fire. A recent review by Bruck *et al.* (2011) on the role of alcohol in residential fire deaths reported the presence of alcohol in 58% of cases, 31% of which had a BAC > 0.20 g/100mL, which is consistent with other studies indicating a higher risk of dying in a fire due to alcohol intoxication [135]. Unfortunately, to the authors' knowledge, no studies have investigated the prevalence of drugs of abuse in fire-related deaths. Indeed, most of the toxicology studies on fire victims are specific to the toxicity of smoke produced from a fire such as inhalation of carbon monoxide and other toxic products of combustion, such as cyanide. One reason for the lack of published toxicological data is the challenging aspect that fire-related deaths present at autopsy. The charring of bodies produced during fires increases the risk of contamination of the toxicological specimens collected at autopsy and limits the availability of routine specimens such as blood and urine. In South Africa, except for carbon monoxide analysis, routine toxicological testing is not performed on fire victims due the poor availability of routine samples. Further research is required to determine the necessity, importance, and significance of routine toxicology analysis of fire-related deaths in South Africa, which constitute a major portion of accidental deaths nationwide [33,36].

Similarly, the role of drugs of abuse in drowning cases is scarcely investigated and/or reported worldwide. However, the information available indicates that alcohol and illicit drug use are contributing factors in deaths due to drowning. For example, Finland has one of the highest reported drowning rates in the developed world [136]. A 1970-2000 comprehensive study by Lunetta *et al.* (2004) found that alcohol was a contributing factor in drowning death for approximately 64% and 52% of boating-related and other types of drowning, respectively. An in-depth analysis of post-mortem cases in the 1998-2000 time period in Finland ($N=704$) indicated that of 84.5% of drowning victims screened positive for ethanol, of which 58% had a BAC ≥ 0.05 g/100mL, 55% had BAC ≥ 0.10 g/100mL, 19% had BAC ≥ 0.25 g/100mL [136]. In addition, 48% of victims screened positive for drugs of abuse other than alcohol; 25.8% tested positive for therapeutic drugs and 20.6% tested positive for a psychotropic drug [136]. Another study conducted in Sweden reported the presence of alcohol (BAC ≥ 0.02 g/100mL) in 38% of drowning victims, with a mean BAC of 0.18 g/100mL [137]. At least one psychoactive substance was detected in 40% of cases with benzodiazepines (21%) as the most common detected drug. Illicit drugs were detected in 10% of victims, with amphetamine (51%) and tetrahydrocannabinol (THC) (41%) as the most frequent illicit substances detected. These psychoactive drugs may influence the risk-taking behaviour and reduce swimming ability in drowning victims [137]. Both studies concluded that the presence of substances (licit and illicit) in drowning is common and may play a part in drowning fatalities. It must be noted that complex toxicological interpretation is required to distinguish the nature of intoxication during drowning. Apart from blood-alcohol analysis, additional toxicological analysis in cases of suspected drowning in South Africa is not routinely requested, and if requested, data are not collated and published. Given the reported findings of drugs and alcohol in cases of drowning, and their possible contributory status to cause of death, it is important that post-mortem toxicological data is obtained and further analysed in South Africa.

IX. Conclusion

Violence in South Africa is the single largest contributor to the burden of injury. The comparative analysis of South Africa's injury mortality with global rates clearly displays the disproportionately larger levels of violence and resulting injury mortality and morbidity. The main manners of violence-related injury fatalities are homicide, suicide, and violent accidents. Specific risk factors, such as alcohol and other drug use, have been reported in the literature to further the understanding of their role in the prevalence and patterns of fatal violence.

An association has been reported between substance use and violence, and numerous studies have examine this relationship in different contexts, in terms of the drugs involved and specific populations of interest. A trend has also been identified in the literature among individuals dying a violent death in which it is common is detect the presence of one or more substances. In South Africa, there is a need for monitoring the post-mortem toxicology of violent deaths to examine the presence and prevalence of substances in these fatalities. The information should then be collated and analysed to further understand the dynamics and patterns of drug use in fatal violence-related cases nationwide.

Research on the nature of drug use and abuse in South Africa has been hampered by the scarcity of data investigating both drugs and violence in the South African society. This review ultimately addresses the potential for post-mortem toxicology investigations as an additional tool in understanding the dynamics between drugs of abuse and violent fatalities in a South African setting. This research may assist in distinguishing injury and violence prevention priorities and required actions directed at high-risk groups, together with identifying possible areas of risk (e.g. socio-environmental) associated with drug abuse and violent death in South Africa, that may require further multi-disciplinary investigation.

X. References

1. Darke S, Degenhardt L, Mattick R. Mortality amongst Illicit Drug Users: Epidemiology, Causes and Intervention. Cambridge: Cambridge University Press; 2006.

2. Dhossche DM, Rich CL, Ghani SO, Isacsson G. Patterns of psychoactive substance detection from routine toxicology of suicides in Mobile, Alabama, between 1990 and 1998. J. Affect. Disord. 2001;64:167–74.

3. City of Cape Town (2014). State of Cape Town Report 2014.

4. Wechsberg WM, Luseno WK, Karg RS, Young S, Rodman N, Myers B, et al. Alcohol, cannabis, and methamphetamine use and other risk behaviours among Black and Coloured South African women: a small randomized trial in the Western Cape. Int. J. Drug Policy. 2008;19:130–9.

5. Peden M, Van Der Spuy J, Smith P, Bautz P. Substance abuse and trauma in Cape Town. South African Med. J. 2000;90:251–5.

6. Darke S, Duflou J, Torok M. Drugs and violent death: Comparative toxicology of homicide and non-substance toxicity suicide victims. Addiction. 2009;104:1000–5.

7. Darke S, Duflou J. Toxicology and circumstances of death of homicide victims in New South Wales, Australia 1996-2005. J. Forensic Sci. 2008;53:447–51.

8. Hedlund J, Ahlner J, Kristiansson M, Sturup J. A population-based study on toxicological findings in Swedish homicide victims and offenders from 2007 to 2009. Forensic Sci. Int. 2014;244:25–9.

9. Darke S, Duflou J, Torok M. Toxicology and circumstances of completed suicide by means other than overdose. J. Forensic Sci. 2009;54:490–4.

10. Goldstein PJ. The Drugs/Violence Nexus: A Tripartite Conceptual Framework. J. Drug Issues. 1985;15:493–506.

11. World Health Organization (WHO). Global status report on violence prevention 2014. [Accessed 2016 Jan 10]. Available from: http://www.who.int/violence_injury_prevention/violence/status_report/2014

12. World Health Organization (WHO). Injuries and Violence: The Facts 2010. Geneva, Switzerland. [Accesed 2016 Jan 5]. Available from: http://www.who.int/violence_injury_prevention/en/

13. Nayar S, Pless IB. Injury Control - A Global View. Inj. Prev. 1997;3:231–2.

14. Mathers C, Fat DM, Boerma JT. The global burden of disease: 2004 update. Geneva, Switzerland: World Health Organization (WHO); 2008 [Accessed 2015 Nov 2]. Available from: www.who.int/evidence/bod

15. Bartolomeos K, Kipsaina C, Grills N, Ozanne-Smith J, Peden M. Fatal injury surveillance in mortuaries and hospitals: a manual for practitioners. Geneva: World Health Organization; 2012.

16. World Health Organization (WHO). Global Health Observatory Data Repository: Cause of death 2008 summary tables. 2008 [Accessed 2015 Apr 7]. Available from: www.who.int

17. District Health Plan 2007-2008. Metro District Health Services (MDHS), Cape Town. [Accessed 2015 Dec 22]. Available from: https://www.capetown.gov.za/en/IDP/Statutory comp plans201011/Annexure_F3_DHP_20072008_v4_Final_151206.pdf

18. Lehola P. How the Count was Done. Statistics South Africa (Statssa). 2012 [Accessed 2016 Jan 8]. p. 1–66. Available from: www.statssa.gov.za

19. Strategic Development Information and GID department. City of Cape Town – 2011 Census. City of Cape Town; 2012 [Accessed 2015 Dec 20]. p. 1–5. Available from: https://www.capetown.gov.za/en/stats/

Pages/Census2011.aspx

20. Western Cape Department of Health. Forensic Pathology Service. [Accessed 2015 Apr 7]. Available from: www.westerncape.gov.za

21. Republic of South Africa. The Inquests Act (Act 58 of 1959) (and its amendments). Pretoria: Government Printer; 1959.

22. Republic of South Africa. National Health Act (Act No. 61 of 2003). Pretoria: Government Printer; 2003.

23. UNODC Global Study on Homicide 2013 (United Nations publication, Sales No. 14.IV.1).

24. Thomson JDS. Coloured homicide trends in South Africa. South African Crime Q. 2004;9–14.

25. South African Police Services (SAPS). An analysis of the national crime statistics 2013/14. [Accessed 2016 Feb 4]. Available from: www.saps.gov.za

26. World Health Organization (WHO). Preventing suicide: A global imperative. Geneva; 2014 [Accessed 2015 Oct 5]. Available from: http://apps.who.int/iris/bitstream/10665/131056/1/9789241564779_eng.pdf?ua=1&ua=1

27. Bertolote J, Fleischmann A. A global perspective in the epidemiology of suicide. Suicidologi. 2002;7:6–8.

28. World Health Organization (WHO). Public health action for the prevention of suicide. 2012 [Accessed 2015 Dec 3]. p. 1–26. Available from: http://www.who.int/mental_health

29. Schlebusch L. Suicide prevention: a proposed national strategy for South Africa. Afr. J. Psychiatry. 2012;15:436–40.

30. Schlebusch L. Current perspectives on suicidal behaviour in South Africa. In: Suffla S, Van Niekerk A, Duncan D, editors. Crime, Violence Inj. Prev. South Africa Dev. Challenges. Tygerberg: Medical Research Council - UNISA Crime, Violence and Injury Lead Programme; 2004. p. 9–21.

31. Matzopoulos R, Norman R, Bradshaw D. The burden of injury in South Africa: Fatal injury trends and international comparisons. In: Suffla S, Van Niekerk A, Duncan N, editors. Crime, Violence Inj. Prev. South Africa Dev. Challenges. Medical Research Council- University of South Africa; 2004. p. 9–21.

32. Bantjes J, Kagee A. Epidemiology of suicide in South Africa: Setting an agenda for future research. South African J. Psychol. 2013;43:238–51.

33. Matzopoulos R, Prinsloo M, Pillay-van Wyk V, Gwebushe N, Mathews S, Martin LJ, et al. Injury-related mortality in South Africa: a retrospective descriptive study of postmortem investigations. Bull. World Health Organ. 2015;93:303–13.

34. Breiding MJ, Wiersema B. Variability of undetermined manner of death classification in the US. Inj. Prev. 2006;12 Suppl 2:ii49–54.

35. Chandran A, Hyder AA, Peek-Asa C. The Global Burden of Unintentional Injuries and an Agenda for Progress. Epidemiol. Rev. 2010;32:110–20.

36. South African Medical Research Council. A profile of fatal injuries in South Africa: Tenth annual report (2008) of the National Injury Mortality Surveillance System. Pretoria, South Africa; 2010.

37. Norton R, Hyder A a, Bishai D, Peden M. Unintentional Injuries. Dis. Control Priorities Dev. Ctries. 2nd ed. World Bank Publications; 2006. p. 737–53.

38. Crocq M-A. Historical and cultural aspects of man's relationship with addictive drugs. Dialogues Clin. Neurosci. 2007;9:355–61.

39. Cornwell A, Cornwell V. Drugs and drug abuse. Drugs, Alcohol Ment. Heal. illustrate. Cambridge University Press; 1993. p. 7–8.

40. World Health Organization (WHO). Management of substance abuse. Psychoactive substances. [Accessed 2016 Jan 26]. Available from: www.who.int

41. World Health Organization. Neuroscience of psychoactive substance use and dependence. Addiction. 2004;99:1361–2.

42. Niesink R, Jaspers RMA, Kornet LMW, van Ree JM. Classification of psychoactive drugs. Drugs Abus. Addict. Neurobehav. Toxicol. illustrate. CRC Press; 1998. p. 40–64.

43. Company DIANE Publishing. DSM-IV and ICD-10 Definitions. Technol. Underst. Prev. Subst. Abus. Addict. illustrate. DIANE Publishing Company; 1996. p. 197–9.

44. Roth J, Finch AJ. Terminology. Approaches to Subst. Abus. Addict. Educ. Communities A Guid. to Pract. that Support Recover. Adolesc. Young Adults. Routledge; 2013. p. 131–2.

45. United Nations Office on Drugs and Crime. World Drug Report 2015 (United Nations publication, Sales No. E.15.XI.6).

46. Parry CDH. Substance Abuse in South Africa : Country Report Focussing On Young Persons. Tygerberg, Cape Town : Medical Research Council (MRC); 1998.

47. Plüddemann A, Dada S, Bhana A, Bachoo S, Perreira T, Freytag K, et al. Monitoring Alcohol & Drug Abuse Trends In South Africa (July 1996-June 2010). SACENDU Reasearch Br. 2010;13.

48. Van Heerden MS, Grimsrud AT, Seedat S, Myer L, Williams DR, Stein DJ. Patterns of substance use in South Africa: results from the South African Stress and Health study. South African Med. J. 2009;99:358–66.

49. UNODC. World Drug Report 2010 (United Nations Publication, Sales No. E.10.XI.13).

50. Dada S, Town C, Burnhams NH, Town C, Williams Y, Town C, et al. Monitoring Alcohol and Drug Abuse Treatment Admissions in South Africa. Cape Town, South Africa; 2013.

51. Pluddemann A, Parry C, Fourie D, Dada S, Bhana A. Alcohol and Drug Abuse Trends: July - December 2009 (Phase 27). SACENDU Reasearch Br. 2010 [Accessed 2015 Nov 27]. Available from: http://www.hsrc.ac.za/en/research-data/view/5191

52. UNODC. World Drug Report 2011 (United Nations Publication, Sales No. E.11.XI.10).

53. Boles SM, Miotto K. Substance abuse and violence: A review of the literature. Aggress. Violent Behav. 2003;8:155–74.

54. Parker RN, Auerhahn K. Alcohol, Drugs, and Violence. Annu. Rev. Sociol. 1998;24:291–311.

55. Lennings CJ, Copeland J, Howard J. Substance use patterns of young offenders and violent crime. Aggress. Behav. 2003;29:414–22.

56. Lavine R. Psychopharmacological treatment of aggression and violence in the substance using population. J. Psychoactive Drugs. 1997;29:321–9.

57. Chermack ST, Blow FC. Violence among individuals in substance abuse treatment: the role of alcohol and cocaine consumption. Drug Alcohol Depend. 2002;66:29–37.

58. Friedman AS. Substance use/abuse as a predictor to illegal and violent behavior. Aggress. Violent Behav. 1998;3:339–55.

59. Hoaken PNSS, Stewart SH. Drugs of abuse and the elicitation of human aggressive behavior. Addict. Behav. 2003;28:1533–54.

60. Darke S, Torok M, Kaye S, Ross J, McKetin R. Comparative rates of violent crime among regular methamphetamine and opioid users: offending and victimization. Addiction. 2010;105:916–9.

61. Garlow SJ. Age, gender, and ethnicity differences in patterns of cocaine and ethanol use preceding suicide. Am. J. Psychiatry. 2002;159:615–9.

62. Kuhns JB, Wilson DB, Maguire ER, Ainsworth SA, Clodfelter TA. A meta-analysis of marijuana, cocaine and opiate toxicology study findings among homicide victims. Addiction. 2009;104:1122–31.

63. Marzuk PM, Tardiff K, Leon AC, Stajic M, Morgan EB, Mann JJ. Prevalence of cocaine use among residents of New York City who committed suicide during a one-year period. Am. J. Psychiatry. 1992;149:371–5.

64. Johnson EM, Belfer ML. Substance abuse and violence: cause and consequence. J. Health Care Poor Underserved. 1995;6:113–21; discussion 121–3.

65. Roth JA. Psychoactive Substances and Violence. Washington, DC: National Institute of Justice, Office of Justice Programs; 1994.

66. Ryan T. Drugs, violence and governability in the future South Africa. Inst. Secur. Stud. Pap. 1997.

67. Copes H, Hochstetler A, Sandberg S. Using a Narrative Framework to Understand the Drugs and Violence Nexus. Crim. Justice Rev. 2015;40:32–46.

68. Oser CB, Mooney JL, Staton-Tindall M, Leukefeld CG. The drugs-violence nexus among rural felony probationers. J. Interpers. Violence. 2009;24:1285–303.

69. Bornman E, Van Eeden R, Wentzel M. Group membership as determinant of violence and conflict: The case of South Africa. Violence South Africa a Var. Perspect. HSRC Press; 1998. p. 85–116.

70. Parry CD, Plüddemann A, Louw A, Leggett T. The 3-metros study of drugs and crime in South Africa: findings and policy implications. Am. J. Drug Alcohol Abuse. 2004;30:167–85.

71. Rivara FP, Mueller BA, Somes G, Mendoza CT, Rushforth NB, Kellermann AL. Alcohol and illicit drug abuse and the risk of violent death in the home. JAMA. 1997;278:569–75.

72. Bradshaw D, Nanna N, Laubscher R, Groenewald P, Joubert J, Nojilana B, et al. South African National Burden of Disease Study 2000: Estimates of Provincial Mortality. Cape Town; 2004.

73. Shabangu T. A comparative inquiry into the nature of violence and crime in Mozambique and South Africa. Pretoria; 2009.

74. Harrison L. The validity of self-reported drug use in survey research: an overview and critique of research methods. NIDA Res. Monogr. 1997;167:17–36.

75. Dhossche DM. Toxicology of suicide: touchstone for suicide prevention? Med. Sci. Monit. 2003;9:SR9–19.

76. Galea S, Ahern J, Tardiff K, Leon AC, Vlahov D. Drugs and firearm deaths in New York City, 1990-1998. J. Urban Health. 2002;79:70–86.

77. Tardiff AKJ, Wallace Z, Tracy M, Piper TM, Vlahov D, Galea S. Drug and alcohol use as determinants of New York City homicide trends from 1990 to 1998. J. Forensic Sci. 2005;50:470–4.

78. Gill JR, Lenz K a, Amolat MJ. Gunshot fatalities in children and adolescents in New York City. J. Forensic Sci. 2003;48:832–5.

79. Tardiff K, Marzuk PM, Leon AC, Hirsch CS, Stajić M, Portera L, et al. Cocaine, opiates, and ethanol in homicides in New York City: 1990 and 1991. J. Forensic Sci. 1995;40:387–90.

80. Chermack ST, Bohnert ASB, Price AM, Austin K, Ilgen MA. Substance use disorders and homicide

death in veterans. J. Stud. Alcohol Drugs. 2012;73:10–4.

81. Kuhns JB, Maguire ER. Drug and alcohol use by homicide victims in Trinidad and Tobago, 2001-2007. Forensic Sci. Med. Pathol. 2012;8:243–51.

82. Garriott JC. Drug use among homicide victims. Changing patterns. Am. J. Forensic Med. Pathol. 1993;14:234–7.

83. Darke S. The toxicology of homicide offenders and victims: A review. Drug Alcohol Rev. 2010;29:202–15.

84. Shaw J, Hunt IM, Flynn S, Amos T, Meehan J, Robinson J, et al. The role of alcohol and drugs in homicides in England and Wales. Addiction. 2006;101:1117–24.

85. Abel EL. Drugs and homicide in Erie County, New York. Int. J. Addict. 1987;22:195–200.

86. Adeagbo BA, Clark C, Collins KA. Homicides committed by youth assailants: a retrospective study. Am. J. Forensic Med. Pathol. 2008;29:219–23.

87. Gill JR, Catanese C. Sharp injury fatalities in New York City. J. Forensic Sci. 2002;47:554–7.

88. Goldstein PJ, Brownstein HH, Ryna PJ, Bellucci PA. Crack and homicide in New York City, 1988 a conceptually based event analysis. Contemp. Drug Probl. 1989;16:651–88.

89. Harruff RC, Francisco JT, Elkins SK, Phillips AM, Fernandez GS. Cocaine and homicide in Memphis and Shelby county: an epidemic of violence. J. Forensic Sci. 1988;33:1231–7.

90. Hood I, Ryan D, Monforte J, Valentour J. Cocaine in Wayne County Medical Examiner's cases. J. Forensic Sci. 1990;35:591–600.

91. Mclaughlin CR, Daniel J, Joost TF. The relationship between substance use, drug selling, and lethal violence in 25 juvenile murderers. J. Forensic Sci. 2000;45:349–53.

92. Rogers C. Gang-related homicides in Los Angeles County. J. Forensic Sci. 1993;38:831–4.

93. Sharps PW, Campbell J, Campbell D, Gary F, Webster D. The role of alcohol use in intimate partner femicide. Am. J. Addict. 2001;10:122–35.

94. Varano SP, McCluskey JD, Patchin JW, Bynum TS. Exploring the drugs-homicide connection. J. Contemp. Crim. Justice. 2004;20:369–92.

95. Varano SP, Cancino JM. An empirical analysis of deviant homicides in Chicago. Homicide Stud. 2001;5:5–29.

96. Welte JW, Abel EL. Homicide: drinking by the victim. J. Stud. Alcohol. 1989;50:197–201.

97. Albrektsen SB, Thomsen JL, Aalund O, Breiting VB, Danielsen L, Helweg-Larsen K, et al. Injuries due to deliberate violence in areas of Denmark. IV. Alcohol intoxication in victims of violence. The Copenhagen Study Group. Forensic Sci. Int. 1989;41:181–91.

98. Allgulander C, Nilsson B. Victims of criminal homicide in Sweden: a matched case-control study of health and social risk factors among all 1,739 cases during 1978-1994. Am. J. Psychiatry. 2000;157:244–7.

99. Lindqvist P. Homicides committed by abusers of alcohol and illicit drugs. Br. J. Addict. 1991;86:321–6.

100. Davies M, Mouzos J. Homicide in Australia: 2005-2006 National Homicide Monitoring Program annual report. Canberra; 2007.

101. Dooley E. Homicide in Ireland 1972-1991. Dublin; 1995.

102. Dooley E. Homicide in Ireland 1992-1996. Dublin; 2001.

103. Lerer LB. Women, homicide and alcohol in Cape Town, South Africa. Forensic Sci. Int. 1992;55:93–9.

104. Duflou JA, Lamont DL, Knobel GJ. Homicide in Cape Town, South Africa. Am. J. Forensic Med. Pathol. 1988;9:290–4.

105. Lunetta P, Penttilä A, Sarna S. The role of alcohol in accident and violent deaths in Finland. Alcohol. Clin. Exp. Res. 2001;25:1654–61.

106. Virkkunen M. Alcohol as a factor precipitating aggression and conflict behaviour leading to homicide. Br. J. Addict. Alcohol Other Drugs. 1974;69:149–54.

107. Hocking MA. Assaults in south east London. J. R. Soc. Med. 1989;82:281–4.

108. Hulse GK, English DR, Milne E, Holman CD. The quantification of mortality resulting from the regular use of illicit opiates. Addiction. 1999;94:221–9.

109. English DR, Holman CDJ, Milne R, Winter MJ, Hulse GK, Bower CI, et al. The quantification of drug-caused morbidity and mortality in australia 1995. Canberra: Commonwealth Department of Human Services and Health; 1995.

110. Leroux LC, Smith LS. Violent deaths and alcoholic intoxication. J. Forensic Med. 1964;11:131–47.

111. Moar JJ. Homicidal penetrating incised wounds of the thorax. An autopsy study of 52 cases. South African Med. J. 1984;65:385–9.

112. Mathews S, Abrahams N, Jewkes R, Martin LJ, Lombard C. Alcohol use and its role in female homicides in the Western Cape, South Africa. J. Stud. Alcohol Drugs. 2009;70:321–7.

113. Bradshaw D, Groenewald P, Laubscher R, Nannan N, Nojilana B, Norman R, et al. Initial burden of disease estimates for South Africa, 2000. South African Med. J. 2003;93:682–8.

114. Reza A, Mercy JA, Krug E. Epidemiology of violent deaths in the world. Inj. Prev. 2001;7:104–11.

115. Dhossche DM, Meloukheia AM, Chakravorty S. The association of suicide attempts and comorbid depression and substance abuse in psychiatric consultation patients. Gen. Hosp. Psychiatry. 2000;22:281–8.

116. Murphy GE. Suicide and Substance Abuse. Arch. Gen. Psychiatry. 1988;45:593.

117. Rich C, Fowler R, Young D. Substance Abuse and Suicide. The San Diego Study. Ann. Clin. Psychiatry. 1989;1:79–85.

118. Mann RE, Zalcman RF, Smart RG, Rush BR, Suurvali H. Alcohol Consumption, Alcoholics Anonymous Membership, and Suicide Mortality Rates, Ontario, 1968-1991. J. Stud. Alcohol. 2006;67:445–53.

119. Wilcox HC, Conner KR, Caine ED. Association of alcohol and drug use disorders and completed suicide: an empirical review of cohort studies. Drug Alcohol Depend. 2004;76 Suppl:11–9.

120. Maloney E, Degenhardt L, Darke S, Mattick RP, Nelson E. Suicidal behaviour and associated risk factors among opioid-dependent individuals: a case-control study. Addiction. 2007;102:1933–41.

121. Harris EC, Barraclough B. Suicide as an outcome for mental disorders. A meta-analysis. Br. J. Psychiatry. 1997;170:205–28.

122. Shields LBE, Hunsaker DM, Hunsaker JC, Ward MK. Toxicologic findings in suicide: a 10-year retrospective review of Kentucky medical examiner cases. Am. J. Forensic Med. Pathol. 2006;27:106–12.

123. Ohberg A, Vuori E, Ojanperä I, Lonngvist J. Alcohol and drugs in suicides. Br. J. Psychiatry. 1996;169:75–80.

124. Centers for Disease Control and Prevention (CDC). Toxicology testing and results for suicide victims--13 states, 2004. MMWR. Morb. Mortal. Wkly. Rep. 2006;55:1245–8.

125. Gunnell D, Ashby D. Antidepressants and suicide: what is the balance of benefit and harm. BMJ. 2004;329:34–8.

126. Reeves RR, Ladner ME. Antidepressant-induced suicidality: an update. CNS Neurosci. Ther. 2010;16:227–34.

127. Marzuk PM, Tardiff K, Leon AC, Hirsch CS, Stajic M, Hartwell N, et al. Use of prescription psychotropic drugs among suicide victims in New York City. Am. J. Psychiatry. 1995;152:1520–2.

128. Coklo M, Stemberga V, Cuculic D, Sosa I, Bosnar A. Toxicology and methods of committing suicide other than overdose. Med. Hypotheses. 2009;73:809–10.

129. Rich CL, Dhossche DM, Ghani S, Isacsson G. Suicide methods and presence of intoxicating abusable substances: some clinical and public health implications. Ann. Clin. Psychiatry. 1998;10:169–75.

130. Dhossche DM, Rich CL, Isacsson G. Psychoactive substances in suicides. Comparison of toxicologic findings in two samples. Am. J. Forensic Med. Pathol. 2001;22:239–43.

131. Haum A, Perbix W, Häck HJ, Stark GB, Spilker G, Doehn M. Alcohol and drug abuse in burn injuries. Burns. 1995;21:194–9.

132. Swenson JR, Dimsdale JE, Rockwell E, Carroll W, Hansbrough J. Drug and alcohol abuse in patients with acute burn injuries. Psychosomatics. 1991;32:287–93.

133. Howland J, Hingson R. Alcohol as a risk factor for injuries or death due to fires and burns: review of the literature. Public Health Rep. 1987;102:475–83.

134. Barillo DJ, Goode R. Substance abuse in victims of fire. J. Burn Care Rehabil. 1996;17:71–6.

135. Bruck D, Ball M, Thomas IR. Fire fatality and alcohol intake: analysis of key risk factors. J. Stud. Alcohol Drugs. 2011;72:731–6.

136. Lunetta P, Smith GS, Penttilä A, Sajantila A. Unintentional drowning in Finland 1970-2000: a population-based study. Int. J. Epidemiol. 2004;33:1053–63.

137. Ahlm K, Saveman B-I, Björnstig U. Drowning deaths in Sweden with emphasis on the presence of alcohol and drugs – a retrospective study, 1992–2009. BMC Public Health. 2013;13:216.

Part C

Publication-ready Manuscript

A Toxicological Study on the Prevalence and Characteristics of Drugs of Abuse in a Cohort of Violent Fatalities in Cape Town, South Africa

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Abstract

Purpose This study analysed the prevalence and characteristics of drugs of abuse in violence-related fatalities to investigate possible associations between drugs of abuse and violent death in Cape Town, South Africa.

Methods Toxicological specimens were collected from victims (*N =104*) of violent homicide, non-overdose suicide, and accidental death at the Salt River mortuary. A qualitative toxicological analysis of these cases was conducted using a targeted screening approach using liquid chromatography coupled to tandem mass spectrometry (AB SCIEX API 3200 Q-TRAP® LC-MS/MS). Additional information on the circumstances of death and demographics of the victims were obtained from post-mortem reports. Detected substances were grouped in major drug classes: benzodiazepines, amphetamines, cannabis, methaqualone, opiates/opioids, cocaine, antidepressants, hallucinogens, and over-the-counter/prescription drugs.

Results Drugs of abuse were detected in 60.6% (*n=63*) of all cases, with more than one drug being detected in 48.1% (*n=50*). Drugs prevalent in homicide cases were pharmaceuticals, amphetamines, methaqualone, and cannabis. Suicide cases were positive for pharmaceuticals, opiates/opioids and antidepressants. Death as a result of violent accidents had opiates/opioids, benzodiazepines, pharmaceuticals, cannabis, and antidepressants present. The combination of diphenhydramine, methamphetamine, and methaqualone was detected in 40% of cases positive for multiple substances. Toxicology data varied by demographic characteristics, manner of death, and drugs present in the victims.

Conclusion The findings of this study support the notion that the presence of substances is associated with violent deaths such as homicide, suicide, and accidents. Routine surveillance of drugs in violent fatalities using toxicological data can help researchers to understand community-specific drug use patterns in relation to the different types of violence-related death in South Africa.

Keywords *Post-mortem forensic toxicology · drugs of abuse · violence · prevalence · violent deaths · Cape Town*

Introduction

An association between drugs of abuse and violence has long been reported in the literature [1–8]. The proposed rationale behind such an association is divided into three different but related mechanisms: (i) the direct psychopharmacological effects of a drug (e.g. disinhibition, intoxication, withdrawal symptoms, paranoia); (ii) the economic-compulsive acts of violence (e.g. robbery); and (iii) the systemic violent environment within illegal drug networks [3,9–12]. Together with other external cross-cutting factors, such as weapon availability; drug abuse increases one's risk of becoming a victim or perpetrator of violence [13]. Alcohol use is the primary substance of abuse which has been correlated with increased risk of violent behaviour and exposure to violent acts, whether perpetrated by a person on oneself or by others on a person (homicide) [14–21]

Drug use and abuse also appears to be associated with violence-related deaths. In fact, licit and illicit drug users exhibit higher all-cause mortality rates compared to the general population [1,3,22–25]. It is further reported that this cohort of individuals commit higher rates of violent crimes, particularly homicides, which in turn constitute a considerable percentage of violent fatalities among substance users [1,24,25]. In an attempt to characterise the drug-violence relationship, several international studies have investigated the presence and role of licit and illicit substances in violent fatalities, particularly in homicide [8,21,26–34] and suicide cases [22,35–42]. Approximately half or more of the victims in these studies tested positive for drugs, and while this may not speak to their intoxication at the time of the fatal incident, researchers do acknowledge the high prevalence of drugs of abuse in victims of violent deaths and have suggested further investigation into the nature and extent of this association.

Drugs most commonly detected in homicide victims, include alcohol followed by psychostimulants (e.g. cocaine), cannabis, and opioids [27,31,32,43,44]. Among suicide victims, pharmaceuticals such as benzodiazepines and antidepressants are most commonly detected [35,38,40,41,45,46]. However, the current reported data on suicides integrates toxicological data from all the different methods of suicide including drug overdose. In this present study, we focus on the toxicology of violence-related deaths, making reference to the involvement of drugs in deaths due to acts of violence. According to Darke's 2009 study on non-overdose suicides, alcohol and pharmaceuticals were also detected in most cases, in comparison to other drugs [42]. The most prevalent illicit drugs detected were cannabis, opioids (such as morphine), and psychostimulants. Given that substance users were reported to use violent means of suicide instead of drug overdose, such toxicological investigations are important to determine patterns in violent fatalities, especially in suicides. Unlike the other types of violent death, research on the toxicology of victims of accidental death is not as extensive and the data available differ across countries and within different regions of the same country [47,48]. While the cause of death may be established in these fatalities, the contribution of intoxication to the cause of death is essential to understanding the nature of these deaths.

South Africa has a longstanding history of violence, with multifaceted complexity lying behind one of the highest rates of injury- and violence-related deaths in the world [49–52]. This is complicated by high rates of illicit substance use and abuse, such as methamphetamine abuse in Cape Town, the provincial capital of the Western Cape Province [53,54]. Given the well-reported toxicological effects of most psychoactive drugs, which depending on the class of drug and the individual to whom it is administered, may include mood and behaviour alteration, stimulation or depression of the central nervous system (CNS), and even increased aggression and psychosis, together with their high potential for abuse, there exists a need to distinguish the role their use plays in violence in South Africa.

As far as the authors are aware, no studies have investigated post-mortem toxicology in victims of violent death within a South African context. This pilot study aimed to investigate the toxicology of drugs of use and abuse, other than alcohol and volatile inhalants, in deaths due to acts of violence in the West Metropole of Cape Town. These fatalities were classified as suspected homicide, non-overdose suicide, and accidental. The objectives were to analyse the prevalence of psychoactive substances in a cohort of deaths by violent means, compare and describe the toxicological findings in the different types of violent death, and examine demographic characteristics, and circumstances of death associated with violence-related fatalities.

Materials and Methods

The South African Setting

The City of Cape Town, the provincial capital of the Western Cape Province of South Africa, has an estimated population of 3.87 million inhabitants spread over eight sub-districts, namely West Metropole, East Metropole, Khayelitsha, Mitchells Plain, North Metropole, South Metropole, and Tygerberg [55,56]. The Salt River mortuary, situated in Cape Town City Bowl Suburb, is a medico-legal facility that serves the West Metropole of Cape Town. The mortuary handles an average case load of more than 3500 autopsies per year [57].

In South Africa, under the Inquests Act of 1959, medico-legal investigations are a statutory requirement for all unnatural deaths, including all deaths that were not due to, or may not have been due to, natural causes [58]. Post-mortem examinations in unnatural deaths are performed by forensic pathologists under the direction of the Forensic Pathology Services (FPS) of the Western Cape Department of Health, so as to determine the cause (disease or injury responsible for the fatal event) and manner (how the cause of death arose) of death. The *suspected* manner of the death is determined by the South African Police Services (SAPS), FPS forensic pathology officers who attend the death scene, and the forensic pathologists during the autopsy. The *final* manner of death is only determined by the magistrate after laboratory analyses and Inquest Court proceedings, which can take several years to complete [59,60].

Toxicological examinations of post-mortem biological samples in the Western Cape are conducted by the Cape Town Forensic Chemistry Laboratory (one of only four Forensic Chemistry laboratories in South Africa). While toxicological testing of biological samples in all unnatural deaths is recommended within the national protocol, it is not routinely performed due to various limitations including insufficient resources associated with training, staff, finance, the large intake of cases, case backlogs, and other challenges currently experienced by the laboratory, mortuary, and related agencies. At the Salt River mortuary, blood is routinely collected for alcohol concentration determination, however, other samples for toxicological analysis are collected at the discretion of the forensic pathologist usually based primarily on the circumstances of death and apparent cause of death at the time of autopsy.

Study Population

The study sample population consisted of 104 adult unnatural death cases, referred to the Salt River FPS between August and October 2015, in which the *apparent* manner of death was homicide, non-overdose suicide, and accidental. Fatalities which fell into HOM (homicide deaths), SUI (non-overdose suicide deaths), and ACC (accidental deaths) groups were included where consent was obtained, apart from some excluded categories outlined below.

All cases of substance-related toxicity such as fatal drug overdose, poisonings due to ingestion of pesticides, acids and other chemicals, as well as gassing by means of carbon monoxide and volatile inhalants were excluded. Cases in which hospital survival precluded the availability of unadulterated post-mortem (blood) specimens (>24-48 hours) and where ante-mortem blood was not available, skeletonized remains, as well as significantly decomposed bodies were also omitted from the study. For research purposes, road traffic accidents were also excluded from the study as they comprise their own separate category of unnatural death in South Africa due to their high prevalence. It is important to note that burns and fire-related deaths were not included due to the poor condition of the toxicological specimens required (and carbon monoxide (CO) and cyanide (CN) were not analysed for). Children, aged younger than 10, were excluded since they are unlikely representative of the general population's drug use patterns.

Qualitative Toxicology Analysis

Chemicals and Reagents

The system suitability test mixture containing amiodarone, amphetamine, caffeine, codeine, diazepam, doxepin, haloperidol, and morphine was provided by Restek (Restek Corp, Bellefonte, PA, USA). All solvents were of LC analytical grade. High-Performance HPLC-grade methanol, acetonitrile, and formic acid were obtained from Merck (Darmstadt, Germany). Ammonium formate obtained from Sigma-Aldrich (Deisenhofen, Germany) was diluted to make a 10mM solution. Deionized water was prepared with a cartridge deionizer from Memtech (Moorenweis, Germany).

Sample Preparation

Femoral blood, heart blood, vitreous humor, urine, bile and hair were obtained at autopsy. Blood samples were collected in grey-top tubes containing sodium fluoride and potassium oxalate, and vitreous humor in red top tubes. Urine and bile were collected in 50mL and 15mL blue top Falcon tubes, respectively. The samples were stored at 4°C until analysis. Hair was collected in an envelope and stored at room temperature. Simple sample preparation procedures were used for screening purposes. Urine samples were prepared using a modified dilute-and-shoot method [6], while the rest of the samples were prepared by using an acetonitrile protein precipitation combined with aqueous dilution method [7]. Hair was not analysed within this study, but collected as part of routine toxicological examination.

LC-MS/MS Targeted Screening of Drugs of Abuse

The LC-MS-MS analysis was performed using a Shimadzu Prominence High Performance Liquid Chromatography (HPLC) system coupled to an AB SCIEX API 3200 Q-TRAP Mass Spectrometer (Applied Biosystems, Foster City, California). A 15-minute gradient was set up using 10 mM ammonium formate for the mobile phase A (MPA) and 50% acetonitrile/50% methanol for the mobile phase B (MPB). The HPLC run consisted of 2% MPB for 1 minute, followed by a linear gradient to 100% MPB between 1-10 minutes, and then holding 100% in MPB for 10-13 minutes, and finally re-equilibrating to 2% MPB for 13-15.5 minutes. The total chromatographic run-time was 15.5 minutes. The total flow rate was 0.6 ml/min. The column oven was set at 40°C. A 30-µl aliquot of the sample was injected.

Samples were analysed using a rapid targeted screening approach, called the AB SCIEX iMethod™ Test for Drugs, which screens for 250 drugs in a single run. Data acquisition and processing was performed with the AB SCIEX Masterview software [61]. A signal-to-noise ratio of 20 is used as cut-off in registering a peak as positive for a particular drug compound. Each peak is also manually verified by the operator. Positive detection also relies on the expected retention time for the drug and its two multiple reaction monitoring transitions.

Qualitative toxicological analysis of all major drug groups (apart from alcohol and volatile inhalants despite being drugs of abuse) was performed on biological specimens (femoral blood, heart blood, urine, vitreous humor, and bile) collected for all violent deaths at autopsy, with no replicates. Hair specimens were not analysed. In this study, toxicological data were reported for the following drug groups (as determined by the detection of the parent drug itself and/or its metabolites): benzodiazepines, amphetamines, cocaine, opiates and opioids, cannabis, methaqualone, hallucinogens, and over-the-counter (OTC)/prescription drugs.

Data Management

Routine information regarding the time and date of death, death scene location, circumstances of death, apparent manner of death, cause of death as well as the decedent's demographic characteristics including age, sex, race, were recorded from post-mortem toxicology specimen collection forms, SAPS and FPS documentation, and autopsy reports from the Salt River mortuary. The South African racial population groups are classified in these reports as Black African, Coloured, Indian, White, or Other, and were used in this study. Permission to analyse the case files was received by the University of Cape Town Human Research Ethics Committee (HREC Ref. 324/2015). All the cases relevant to this study were reviewed by the researchers. The compiled information was then securely stored in an anonymous and controlled access database.

Statistical Analysis

Counts and percentages were tabulated for demographics, circumstances related to death, and post-mortem toxicological results. Statistical significance testing was conducted using the Fisher's exact test and a test of (equality) proportions at a 95% significance level. In this study, group comparisons specifically refers to statistical significant differences between the HOM group and Others (SUI and ACC groups were combined due to the small sample size). All analyses were conducted using STATA14 [62].

Ethical Considerations

This study was approved by the University of Cape Town Human Research Ethics Committee (HREC Ref. 324/2015). Written informed consent was obtained from the next-of-kin of each decedent. All the information used in the study remained anonymous and was kept confidential throughout the study duration.

Results

Cases

Of a total of 104 violent deaths (Table 1), 99 were male (95.2%) and 5 were female (4.8%). The overall mean age of victims was 31 years (SD=12), ranging between 10-75 years. Over two-thirds (*n* = 69; 66.3%) of the victims documented in the study were Black African, approximately one-third were Coloured (*n* = 30; 28.8%), and nearly 5% (*n* = 5) were White. Of the 104 fatalities recorded in the study period, 94 were homicides (90.4%), 5 were suicides (4.8%), and 5 were accidents (4.8%). Overall, deaths were mostly attributed to gunshot wounds (*n* = 46; 44.2%), stabbings (*n* = 28; 26.9%), or assaults (*n* = 18; 17.3%). Less frequent causes of death were hanging (*n* = 5; 4.8%), drowning (*n* = 3; 2.9%), multiple causes (e.g. assault-stab, shot-stab cases) (*n* = 2; 1.9%), electrocution (*n* = 1; <1%), and 'freak accidents' (*n* = 1; <1%) – events occurring under highly unusual and unlikely circumstances.

Of all 94 homicides, 92 (97.9%) were male and 2 (2.1%) were female, 64 (68.1%) were Black African, 28 (29.8%) were Coloured, and 2 (2.1%) were White. The average age of homicide victims was 29.5 years old. The leading cause of death among homicides were gunshot injuries (*n* = 46; 49%), followed by stabbing (*n* = 28; 29.8%), assault (*n* = 18; 19.1%), and multiple causes (*n* = 2; 2.1%).

Suicides comprised 4.8% of all recorded violent deaths in the study, and of whom 3 were male, 2 were female, 2 were Black African, 1 was white, and 2 were Coloured. The mean age of suicide victims was 44.8 years old, and the apparent cause of death in all five cases was hanging.

Of all five accidental violent deaths (4.8% of total violent deaths) included in the study, 4 were male, 1 was female, 3 were Black African, 1 was Coloured and 1 was White. The average age for victims of accidental violent death was 35.8 years old. The apparent cause of death for these accidents were drowning (*n* = 3), electrocution (*n* = 1), and freak accidents (*n* = 1). For any statistical test conducted, the SUI and ACC cases were pooled together as 'Others' and compared with the HOM group.

Table 1 Demographic and case characteristics of the 104 study cases.

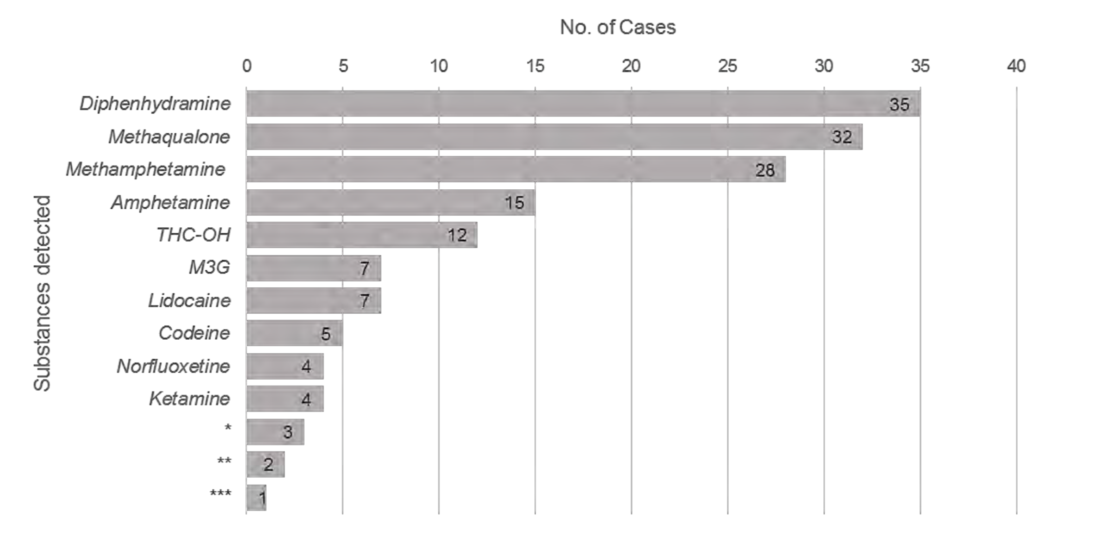
Victim Characteristics		HOM (*n=94*)	SUI (*n=5*)	ACC (*n=5*)	Total (*N=104*)
		(n) / % of the total no. of cases			
Mean age (years)		29.5	44.8	35.8	30.6
Gender	*Male*	(92) / 88.5	(3) / 2.9	(4) / 3.8	(99) / 95.2
	Female	(2) / 1.9	(2) / 1.9	(1) / 0.96	(5) / 4.8
Ethnicity	*Black African*	(64) / 61.5	(2) / 1.9	(3) / 2.9	(69) / 66.3
	Coloured	(28) / 26.9	(1) / 0.96	(1) / 0.96	(30) / 28.8
	White	(2) / 1.9	(2) / 1.9	(1) / 0.96	(5) / 4.8
	Indian	-	-	-	-
	Other	-	-	-	-
Cause of death	*Gunshot*	(46) / 44.2	-	-	(46) / 44.2
	Stabbing	(28) / 26.9	-	-	(28) / 26.9
	Assault	(18) / 17.3	-	-	(18) / 17.3
	Hanging	-	(5) / 4.8	-	(5) / 4.8
	Drowning	-	-	(3) / 2.9	(3) / 2.9
	Electrocution	-	-	(1) / 0.96	(1) / 0.96
	Freak	-	-	(1) / 0.96	(1) / 0.96
	Multiple	(2) / 1.9	-	-	(2) / 1.9

Post-mortem Toxicology

Targeted LC-MS/MS screening for drugs of abuse was performed on all 104 collected cases. A total of 42 different substances were detected in various biological specimens and categorized into the following drug groups: benzodiazepines, amphetamines, cocaine, opiates and opioids, cannabis, methaqualone, hallucinogens, antidepressants, and OTC and/or prescription drugs (Table 2). Figure 1 illustrates the proportional distribution of the different substances detected in all 104 cases with diphenhydramine, methaqualone, and methamphetamine, as the three most predominant drug findings, present in 35 (33.7%), 32 (30.8%), and 28 (26.9%) cases, respectively, with some cases testing positive for more than one analyte.

Table 2 List of detected substances categorised into drug groups.

Major Drug Groups Detected	Specific Substances Detected
Benzodiazepines	Diazepam, Nordiazepam, Temazepam, Midazolam
Amphetamines	Methamphetamine, Amphetamine, 3,4-methylenedioxymethamphetamine (MDMA), Methcathinone
Cocaine	Cocaine, Benzoylecgonine
Opiates and Opioids	Morphine, Hydromorphone, Morphine 3-β-D-glucuronide (M3G), Codeine, Tramadol, Fentanyl, Norfentanyl
Cannabis	11-Hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC), 11-nor-9-Carboxy-THC (THC-COOH)
Hallucinogens	Ketamine, Phencyclidine (PCP)
Methaqualon	Methaqualone
Antidepressants	Desipramine, Clomipramine, Fluoxetine, Norfluoxetine, Protriptyline, Nortriptyline
OTC, Prescription, and Other Drugs	Diphenhydramine, Atropine, Metformin, Amlodipine, Acetaminophen, Sibutramine, Atenolol, Lidocaine, Diltiazem, Dexamethasone, Reserpine, Ranitine, Salicylamide, Doxylamide



* Nordiazepam, Diltiazem, THC-COOH
** Diazepam, Temazepam, MDMA, Cocaine, Morphine, Acetaminophen, Metformin, Ranitine
*** Midazolam, Methcathinone, Benzoylecgonine, Hydromorphone, Tramadol, Fentanyl, Norfentanyl, PCP, Desipramine, Clomipramine, Protriptyline, Nortriptyline, Fluoxetine, Atropine, Amlodipine, Dexamethasone, Reserpine, Salicylamide, Sibutramine, Atenolol, Doxylamide

Fig. 1 Distribution of the 42 substances detected in all 104 violent death cases. Multiple substances were detected in 50 cases.

Comparative Toxicology

Psychoactive substances were detected in 60.0% (n = 63) of cases and with more than one drug being detected in almost half ($n = 50$, 48.1%) of the total cases (Table 3). There was no significant group differences between HOM and Others (SUI+ACC) in the type of substances detected. Overall, the substances detected were part of the following groups: the OTC/Prescription drugs (n = 46; 44.2%), followed by amphetamines (n = 33; 31.7%), methaqualone (n = 32; 30.8%), cannabis (n = 15; 14.4%), opiates/opioids (n = 15; 14.4%), benzodiazepines (n = 8; 7.7%), antidepressants (n = 6; 5.8%), hallucinogens (n = 5; 4.8%), and cocaine (n = 2; 1.9%). For each of the drug groups, detection of substances was more frequent among HOM cases. In terms of group comparisons (HOM vs. others), the detection of drugs in homicides was significant for amphetamines ($\rho = 0.028$) and methaqualone ($\rho = 0.029$) only, accounting for 31.7% and 30.8% of all cases. SUI cases tested positive for OTC/prescription drugs (n = 2; 1.9%), antidepressants (n = 1; 0.96%) such as desipramine, and opiates/opioids (n = 1; 0.96%) such as codeine. Drugs present in ACC cases were opiates/opioids (n = 2; 1.9%), benzodiazepines (n = 1; 0.96%), OTC/prescription drugs (n = 1; 0.96%), antidepressants (n = 1; 0.96%), and cannabis (n = 1; 0.96%).

Multiple Substances

Substances were present in 63 cases, 50 of which had multiple substances present. Table 4 shows the toxicology data of each cases in which more than one substance was detected. As mentioned above, the three most prevalent drugs in these 104 violent deaths were diphenhydramine (n = 35; 33.7%), methaqualone (n = 32; 30.8%), and methamphetamine (n = 28; 26.9%), and 34 (68%) of cases with multiple substance present had two or more of these three drugs present (Figure 1). Table 4 also illustrates the counts of cases in various drug combinations between these three substances: (I+II), diphenhydramine and methamphetamine; (I+III), diphenhydramine and methaqualone; (II+III), methamphetamine and methaqualone; and (I+II+III), diphenhydramine, methamphetamine, and methaqualone. Of all 50 violent deaths positive for multiple substances, 5 (10%) cases had diphenhydramine and methamphetamine present, 8 (16%) had diphenhydramine combined with methaqualone, 1 (5%) had methamphetamine and methaqualone, and 20 (40%) had all three drugs present.

Toxicology and Cause of Death

Table 5 provides the proportional distribution of drug groups present in the different causes and manners of death. The most common substances detected in gunshot-related homicides were OTC/prescription drugs (n = 22) with 18 cases positive for diphenhydramine, methaqualone (n = 18) and amphetamines (n = 16). In stabbing-related cases, drugs detected included OTC/prescription drugs (n = 10; 6 cases positive for diphenhydramine), amphetamines (n = 7), and cannabis (n = 5). OTC/prescription drugs (n = 11) with 10 cases positive for diphenhydramine, methaqualone (n = 9) and amphetamines (n = 9) were detected in assault cases and amphetamines (n = 1) and methaqualone (n = 1) in homicides due to multiple causes. The substances detected in all five cases of suicides were OTC/prescription drugs (n = 2), antidepressants (n = 1), and opiates/opioids (n = 1). In accidental deaths, drugs were detected in drowning only: opiates/opioids (n = 2), benzodiazepines (n = 1), cannabis (n = 1), antidepressants (n = 1), and OTC/prescription drugs (n = 1).

Table 3 Comparative toxicology of psychoactive substances in all violent deaths.

Violent Deaths	HOM (*n=94*)	SUI (*n=5*)	ACC (*n=5*)	Total (*N=104*)	HOM vs. Others (Fisher's Exact Test)
	(n) / % of the total no. of cases				
Overall Toxicology					
Substances detected	(58) / 55.8	(3) / 2.9	(2) / 1.9	(63) / 60.6	$\rho = 0.510$
Multiple Substances	(47) / 45.2	(2) / 1.9	(1) / 0.96	(50) / 48.1	$\rho = 0.323$
Specific Drug Groups					
Benzodiazepines	**(7) / 6.7**	**-**	**(1) / 0.96**	**(8) / 7.7**	$\boldsymbol{\rho = 0.568}$
Nordiazepam	(3) / 2.9	-	-	(3) / 2.9	$\rho = 1.000$
Diazepam	(2) / 1.9	-	-	(2) / 1.9	$\rho = 1.000$
Temazepam	(2) / 1.9	-	-	(2) / 1.9	$\rho = 1.000$
Midazolam	(1) / 0.96	-	-	(1) / 0.96	$\rho = 1.000$
Amphetamines	**(33) / 31.7**	**-**	**-**	**(33) / 31.7**	$\boldsymbol{\rho = 0.028}$ *
Methamphetamine	(28) / 26.9	-	-	(28) / 26.9	$\rho = 0.059$ *
Amphetamine	(15) / 14.4	-	-	(15) / 14.4	$\rho = 0.351$
MDMA	(2) / 1.9	-	-	(2) / 1.9	$\rho = 1.000$
Methcathinone	(1) / 0.96	-	-	(1) / 0.96	$\rho = 1.000$
Cocaine	**(2) / 1.9**	**-**	**-**	**(2) / 1.9**	$\boldsymbol{\rho = 1.000}$
Opiates/Opioids	**(12) / 11.5**	**(1) / 0.96**	**(2) / 1.9**	**(15) / 14.4**	$\boldsymbol{\rho = 0.156}$
Morphine 3-β-D-glucuronide	(7) / 6.7	-	-	(7) / 6.7	$\rho = 1.000$
Codeine	(3) / 2.9	(1) / 0.96	(1) / 0.96	(5) / 4.8	$\rho = 0.072$ *
Morphine	(2) / 1.9	-	-	(2) / 1.9	$\rho = 1.000$
Hydromorphone	(1) / 0.96	-	-	(1) / 0.96	$\rho = 1.000$
Tramadol	-	-	(1) / 0.96	(1) / 0.96	$\rho = 0.096$
Fentanyl	-	-	(1) / 0.96	(1) / 0.96	$\rho = 0.096$
Norfentanyl	(1) / 0.96	-	-	(1) / 0.96	$\rho = 1.000$
Cannabis	**(14) / 13.5**	**-**	**(1) / 0.96**	**(15) / 14.4**	$\boldsymbol{\rho = 1.000}$
THC-OH	(11) / 10.6	-	(1) / 0.96	(12) / 11.5	$\rho = 1.000$
THC-COOH	(3) / 2.9	-	-	(3) / 2.9	$\rho = 1.000$
Methaqualone	**(32) / 30.8**	**-**	**-**	**(32) / 30.8**	$\boldsymbol{\rho = 0.029}$ *
Hallucinogens	**(5) / 4.8**	**-**	**-**	**(5) / 4.8**	$\boldsymbol{\rho = 1.000}$
Ketamine	(4) / 3.8	-	-	(4) / 3.8	$\rho = 1.000$
PCP	(1) / 0.96	-	-	(1) / 0.96	$\rho = 1.000$
Antidepressants	**(4) / 3.8**	**(1) / 0.96**	**(1) / 0.96**	**(6) / 5.8**	$\boldsymbol{\rho = 0.102}$
Norfluoxetine	(3) / 2.9	-	(1) / 0.96	(4) / 3.8	$\rho = 0.337$
Fluoxetine	-	-	(1) / 0.96	(1) / 0.96	$\rho = 0.096$
Desipramine	-	(1) / 0.96	-	(1) / 0.96	$\rho = 0.096$
Clomipramine	(1) / 0.96	-	-	(1) / 0.96	$\rho = 1.000$
Protriptyline	-	-	(1) / 0.96	(1) / 0.96	$\rho = 0.096$
Nortriptyline	-	-	(1) / 0.96	(1) / 0.96	$\rho = 0.096$
OTC/Prescription Drugs	**(43) / 41.3**	**(2) / 1.9**	**(1) / 0.96**	**(46) / 44.2**	$\boldsymbol{\rho = 0.506}$
Diphenhydramine	(34) / 32.7	(1) / 1.9	-	(35) / 33.7	$\rho = 0.159$
Lidocaine	(7) / 6.7	-	-	(7) / 6.7	$\rho = 1.000$
Diltiazem	(2) / 1.9	-	(1) / 0.96	(3) / 2.9	$\rho = 0.264$
Acetaminophen	(1) / 0.96	(1) / 0.96	-	(2) / 1.9	$\rho = 0.184$
Atropine	(1) / 0.96	-	-	(1) / 0.96	$\rho = 1.000$
Metformin	(1) / 0.96	(1) / 0.96	-	(2) / 1.9	$\rho = 0.184$
Amlodipine	(1) / 0.96	(1) / 0,96	-	(2) / 1.9	$\rho = 0.184$
Atenolol	(1) / 0.96	-	-	(1) / 0.96	$\rho = 1.000$
Doxylamine	-	(1) / 0.96	-	(1) / 0.96	$\rho = 0.096$
Dexamethasone	-	-	(1) / 0.96	(1) / 0.96	$\rho = 0.096$
Ranitine	(2) / 1.9	-	-	(2) / 1.9	$\rho = 1.000$
Reserpine	(1) / 0.96	-	-	(1) / 0.96	$\rho = 1.000$
Salicylamine	(1) / 0.96	-	-	(1) / 0.96	$\rho = 1.000$
Sibutramine	(1) / 0.96	-	-	(1) / 0.96	$\rho = 1.000$

*Statistically significant

Table 4 Cases positive for multiple substances with the combination of the three most prevalent drugs detected.

ID	Drugs present in cases tested positive for 'multiple substances'	Combinations (I, II, III)
1	Diazepam, Nordiazepam	
2	Methamphetamine, Amphetamine	
4	Diphenhydramine, Methamphetamine, Amphetamine, THC-COOH	I+II
9	Cocaine, Benzoylecgonine, Atropine,	
14	Diphenhydramine, Methamphetamine, Amphetamine, Methaqualone	I+II+III
15	Diphenhydramine, Methamphetamine, Methaqualone	I+II+III
17	Diphenhydramine, Amphetamine, Methaqualone, Morphine, Hydromorphone	I+II+III
21	Diphenhydramine, Methamphetamine, Amphetamine, Methaqualone, Morphine-3-β-D-glucuronide	I+II+III
23	Diphenhydramine, Methamphetamine, Amphetamine, Methaqualone, Morphine-3-β-D-glucuronide	I+II+III
24	Metformin, Amlodipine	
30	Diphenhydramine, Methamphetamine, Amphetamine	I+II
36	Diphenhydramine, Methaqualone, THC-COOH	I+III
40	Diphenhydramine, Methaqualone, Atenolol, Clomipramine	I+III
41	THC-OH, Lidocaine, Norfluoxetine	
42	Methaqualone, Norfluoxetine THC-OH, Nordiazepam, Morphine-3-β-D-glucuronide	
43	Diphenhydramine, Methamphetamine, MDMA, Temazepam, Diltiazem, THC-OH	I+II
44	Diphenhydramine, THC-OH, Methaqualone, Norfluoxetine, Lidocaine	I+III
45	THC-OH, Fluoxetine, Protriptyline, Nortriptyline, Norfluoxetine, Tramadol, Nordiazepam, Diltiazem, Fentanyl, Dexamethasone	
46	Diphenhydramine, Methaqualone, THC-OH, Nordiazepam, Reserpine, Ranitine	I+III
48	Diphenhydramine, Methamphetamine, Methaqualone, THC-OH	I+II+III
49	Lidocaine, THC-OH, Ketamine, Norfentanyl, Temazepam, Diltiazem	
50	Diphenhydramine, Methaqualone, MDMA, THC-OH	I+III
54	Diphenhydramine, THC-COOH	
56	Amphetamine, Codeine	
59	Diphenhydramine, Methamphetamine, Amphetamine, Methaqualone, Diazepam, Morphine-3-β-D-glucuronide	I+II+III
60	Amphetamine, Codeine	
62	Methcathinone, PCP, Codeine	
63	Diphenhydramine, Methamphetamine, Amphetamine, Methaqualone	I+II+III
64	Metformin, Amlodipine	
65	Diphenhydramine, Methamphetamine, Methaqualone, Salicylamide, Morphine-3-β-D-glucuronide	I+II+III
66	Acetaminophen, Codeine, Diphenhydramine, Doxylamine	
67	Diphenhydramine, Methamphetamine, Amphetamine, Methaqualone, Morphine, Morphine-3-β-D-glucuronide	I+II+III
70	Diphenhydramine, Methamphetamine, Amphetamine, Methaqualone, Cocaine, Ranitine, Morphine-3-β-D-glucuronide	I+II+III
72	Diphenhydramine, Methamphetamine, Methaqualone	I+II+III
73	Diphenhydramine, Methamphetamine, Methaqualone	I+II+III
74	Diphenhydramine, Methamphetamine, Methaqualone	I+II+III
75	Diphenhydramine, Methamphetamine	I+II
76	Midazolam, Lidocaine	
77	Diphenhydramine, Methamphetamine, Methaqualone	I+II+III
80	Diphenhydramine, Methaqualone	I+II
84	Diphenhydramine, Methamphetamine, Methaqualone	I+II+III
86	Diphenhydramine, Methamphetamine, Methaqualone	I+II+III
90	Lidocaine, Ketamine	
91	Diphenhydramine, Methaqualone, THC-OH	I+II
95	Diphenhydramine, Methamphetamine, Methaqualone	I+II+III
98	Diphenhydramine, Methamphetamine, Methaqualone, THC-OH	I+II+III
100	Diphenhydramine, Methamphetamine, Methaqualone	I+II+III
101	Diphenhydramine, Methaqualone	I+II
102	Methamphetamine, Methaqualone	II+III
103	Diphenhydramine, Methamphetamine	I+II

I, Diphenhydramine; II, Methamphetamine; III, Methaqualone.

Table 5 Proportion of drug groups in different apparent causes of death.

Major Drug Classes	HOM				SUI	ACC			Total (*N=104*)
	Gunshot (*N=46*)	Stabbing (*N=28*)	Assault (*N=18*)	Multiple (*N=2*)	Hanging (*N=5*)	Drowning (*N=3*)	Electrocution (*N=1*)	Freak (*N=1*)	
	(n) / % of total no. of cases								
Benzodiazepines	(5) / 4.8	(1) / 0.96	(1) / 0.96	-	-	(1) / 0.96	-	-	(8) / 7.7
Amphetamines	(16) / 15.4	(7) / 6.7	(9) / 8.7	(1) / 0.96	-	-	-	-	(33) / 31.7
Cocaine	(1) / 0.96	-	(1) / 0.96	-	-	-	-	-	(2) / 1.9
Opiates/Opioids	(7) / 6.7	(3) / 2.9	(2) / 1.9	-	(1) / 0.96	(2) / 1.9	-	-	(15) / 14.4
Cannabis	(6) / 5.8	(5) / 4.8	(3) / 2.9	-	-	(1) / 0.96	-	-	(15) / 14.4
Methaqualone	(18) / 17.3	(4) / 3.8	(9) / 8.7	(1) / 0.96	-	-	-	-	(32) / 30.8
Hallucinogens	-	(4) / 3.8	(1) / 0.96	-	-	-	-	-	(5) / 4.8
Antidepressants	(2) / 1.9	(1) / 0.96	(1) / 0.96	-	(1) / 0.96	(1) / 0.96	-	-	(6) / 5.8
OTC/Prescription Drugs and Others	(22) / 21.2	(10) / 9.6	(11) / 10.6	-	(2) / 1.9	(1) / 0.96	-	-	(46) / 44.2

Discussion

This preliminary research study reports the first prospective and comparative post-mortem toxicology data on drugs of abuse in violent deaths (suicide, homicide, and accidents) in Cape Town, South Africa. The study is in accord with previous research that psychoactive substances are commonly detected in victims of violent death [27,31–33,38–44]. Additionally, with almost half of the cases positive for the detection of more than one drug, the findings support the existence of an association between substance use and violent death, which requires further investigation. Interestingly, the three most commonly detected drugs or drug groups, diphenhydramine, methamphetamine, and methaqualone, are consistent with the drug use epidemiology of Cape Town and fall under the five most widely used drugs in the entire district [63]. The results suggest a high prevalence of drugs of abuse in the cohort of violent deaths studies. This indicates the necessity of toxicological investigation in these cases to distinguish the nature of contribution of intoxication to death.

Cases

In this study, nearly all of the decedents were male. This correlates with Darke's 2009 findings in which over three-quarter (78.4%) of victims of violent death were males [33]. Compared with the other death groups in the study, homicides involving males, accounting for 88.5% of all deaths, were significantly higher ($\rho = 0.006$) than female homicides (Table 1). Consistent with a recent national study on violence- and injury-related fatalities, male mortality rates are consistently and significantly higher, with the highest male to female mortality ratios in homicides occurring in metropolitan regions of South Africa [50]. Interestingly, Connor *et al.* (2013) in Colorado, United States, examined the gender differences in the presence of substances in violent suicides and homicides and found that drugs associated with homicide (cannabis, amphetamines, and cocaine) are more prevalent in males, while substances (opiates and antidepressants) related to suicide are more common among females [64]. In this study, amphetamines, cannabis and methaqualone were found to be associated with male homicide, while antidepressants and opiates/opioids were present in female suicide victims (none in male suicide victims) (Part D, Appendix A, Table 1). It is important to note however, that the low sample size of the study limits further discussion. The high male predominance in violent deaths found in this study requires further investigation to understand underlying circumstances and monitor future trends in gender-based violence that results in death.

Of all 104 violent deaths, 66.3% (*n=69*) of the victims belong to the Black African race and most of them (*n*=64, 92.8%) died in homicidal circumstances. This may suggests that Black African individuals are more likely to die a violent death, particularly in the form of homicide, than any other population group. However, given the fact that consent was obtained prospectively in this study, several cases were either lost or permission to collect samples was denied by the decedent's next-of-kin. According to Matzopoulos *et al.* (2015), Black African and Coloured victims of violence- and injury-related deaths had comparable rates of homicide in South Africa, 2009, with the highest risk of homicide among the Coloured population group [50]. However, in metro areas, homicide rates were highest in Black African individuals, and in non-metro areas, they were highest in Coloured individuals [50]. Due to several limitations in this study, further discussion in terms of racial differences in violent fatalities in Cape Town is restricted and needs a larger sample size for future research.

Post-mortem Toxicology

Among the 42 substances detected in this study, the three drugs most commonly present in these 104 cases of violent death were diphenhydramine, methaqualone, and methamphetamine. Diphenhydramine is a first generation antihistamine that blocks the effects of histamine, a natural chemical produced in the body during an allergic reaction. It is used to relieve symptoms of allergy such as rashes, itchiness, watery eyes, runny nose, and sneezing. Given that diphenhydramine is mostly detected simultaneously in combination with methamphetamine and methaqualone, the high prevalence of diphenhydramine obtained in this study is indicative of it being either an adulterant used to "dilute" a drug, to increase the quantity of the drug, to reduce manufacturing costs, and/or to change the drug potency to produce a desired effect when taken together. Methaqualone, known as mandrax (usually mixed with cannabis) in South Africa, is a synthetic, barbiturate-like, anxiolytic, and sedative-hypnotic drug. It was originally medically prescribed to treat insomnia and anxiety due to its CNS depressant properties. Methaqualone increases the activity of gamma-aminobutyric acid (γ-Aminobutyric acid or GABA) receptors, which then reduces blood pressure, breathing rate, and heart rate, leading to a state of deep relaxation. Owing to its high potential for abuse, methaqualone rapidly became a popular recreational drug and its use was banned in several countries around the world.

In South Africa, the mandrax tablet, in which the main ingredient is methaqualone, is usually crushed and combined with cannabis in various ways: "white pipe" – cannabis mixed with mandrax and smoked in a cannabis pipe [65]; "cremora" – finely ground mandrax sprinkled on cannabis; and "double barrel" – cannabis mixed with two crushed mandrax tablets [66]. Mandrax was originally manufactured with diphenhydramine (250mg methaqualone/25mg diphenhydramine). Therefore, the detection of methaqualone-diphenhydramine drug combination in this study indicates that the clandestine market is likely to be using similar preparation methods as it is reported to produce a greater sedative/hypnotic effect than methaqualone alone [67]. Smoking mandrax with cannabis produces a greater "rush", the effects are similar to other depressants and last for several hours as methaqualone tends to enhance the main active component of cannabis (Δ^9-THC). Therefore, the results of the study on the detection of multiple substances correlate to the use of cannabis and methaqualone, as well as other sedatives such as benzodiazepines with methaqualone. This together with the presence of diphenhydramine may suggest that drug users seek greater hypnotic-sedative effects than those produced by methaqualone alone. However, this needs to be further correlated to the quantitation in varying biological specimens and further investigation into the drug profiling of mandrax tablets/powder is required.

Known as tik in South Africa, methamphetamine is a highly addictive CNS stimulants. Methamphetamine is illegally trafficked and sold as a euphoriant and aphrodisiac drug mainly for recreational use. Medically, methamphetamine is used in low doses in the treatment of narcolepsy and attention deficit disorders. The drug can increase alertness, increase brain function, and elevate the mood of the drug user. Methamphetamine abuse can induce psychosis, unpredictable behaviours, mood alterations, and delusions. It is metabolized to amphetamine (active), p-OH-amphetamine and norephedrine (both inactive). The high prevalence of methamphetamine detected in violent fatalities is consistent with the methamphetamine epidemiology of South Africa, which is increasing at alarming rates, especially within the Cape Town area [53,63,68,69].

Comparative Toxicology

An important observation from this post-mortem toxicological study is the notable differences between the toxicology of violent homicide, non-overdose suicide, and accidental cases. Psychoactive substances most frequently detected in the HOM group were amphetamines (primarily methamphetamine), methaqualone, and cannabis. Due to the cultural significance and pharmacological nature of psychostimulants such as amphetamines, they are more likely to be present in homicide victims [3,33,64]. Various studies in the literature have recognised and discussed the association between the use of amphetamines, increased aggression and violent behaviour [2,3,54,70]. This study's findings are therefore consistent with similar comparative studies in which amphetamines, such as methamphetamine, are more prevalent in homicide victims than suicide victims [33,64]. Similarly, consistent with other reports, cannabis was also found to be more prevalent in homicide victims [33,34,71,72]. However, unlike methamphetamine, cannabis is less likely to induce violent behaviour due to its sedating effects. Some studies found that low doses of cannabis can momentarily inhibit violent and aggressive behaviour, hence depressing CNS activity in general [2]. When taken in large amounts, cannabis can have psychoactive properties similar to hallucinogens which initiates paranoia, fear, anxiety, and psychosis, which can potentially result in an aggressive outbreak [73]. Moreover, cannabis is one the major drugs illicitly produced and trafficked around the world, which implies that substance users have a greater risk of exposure to economic-compulsive and systemic violence [74]. The context of presence of these drugs together requires further interpretation. For example, the use of cannabis may assist in alleviating the 'come-down' from a methamphetamine high.

Unique to this study, homicide victims were more likely to test positive for methaqualone. This correlates with the World Drug Report finding that methaqualone use has become concentrated in South Africa [74]. As mentioned above, methaqualone is a synthetic drug with sedative and hypnotic effects. However, the drug possesses various side effects such as euphoria, psychosis, and physical and psychological dependency, which may increase the risk of systemic violence. A South African study conducted in the late 90's by Parry *et al.* (2005) found a high prevalence of methaqualone use among trauma patients, especially in Cape Town, and recognised the need to further research the underlying causes of such trends [75]. Likewise, in a 2004 study investigating the drug-crime nexus in South Africa, Mandrax was found to be the second most commonly detected drug among arrestees, hence reflecting the distinctive drug market established in this country [52]. The extent to which the mandrax combinations directly or indirectly encourage violent behaviour is unknown. Again, the possible use of these drugs together needs to be contextualised further within the boundaries of post-mortem toxicological information and inferences.

Psychoactive substances present in the SUI cases – non-overdose suicides due to violent acts – were antidepressants and opiates/opioids, such as desipramine and codeine, respectively. This is consistent with two previous studies conducted on the toxicology of suicide by means other than overdose in which pharmaceuticals (benzodiazepines, antidepressants, psychotics) and opioids were most commonly detected [33,42]. In clinical grounds, antidepressants are used to treat symptoms of depression. However, some researchers claim that antidepressants have paradoxical effects which may aggravate depression [46]. Although the relationship between antidepressants and suicide risk still remains unclear, numerous studies reports the presence of these drugs in suicide victims [35,38]. Opiates/opioids compounds relieve pain and produce sedation. There is currently no evidence of an association between the use of opiates/opioids intoxication and violence [2]. As they tend to depress brain activity, opiates/opioids are unlikely to be involved in any form of fatal violence while intoxicated. Given that pharmaceutical drugs, such as antidepressants and opiates/opioids, are prescribed substances for medical use, their presence in suicide victims who died from a violent death and not from a drug overdose may be a manifestation of psychopathology in these individuals who may be susceptible to suicide (e.g. antidepressants - mood disorders) [33]. For instance, in one suicide case, desipramine was detected in the urine sample. Interestingly, Darke *et al.* (2007, 2009) reports that the toxicology of suicides reveal that the use of suicide methods other than drug overdose are more common among drug users [24,42]. Due to the low study population size, we are unable to investigate this correlation in this study. This study does indicate however, that further research is required to investigate the identified possible association between drug use and methods of suicidal death in a South Africa context.

Multiple Substances

The presence of more than one substance was detected in 50 (48.1%) cases overall, of whom 40% (*n=20*) had a combination of diphenhydramine, methamphetamine, and methaqualone. The drug combination of diphenhydramine-methaqualone was detected more frequently than diphenhydramine-methamphetamine and methamphetamine-methaqualone. This may depend upon the findings in particular biological samples and the pharmacokinetic and pharmacodynamics characteristics of the drugs involved. The use and abuse of methaqualone-diphenhydramine drug combination has been reported to be associated with physiological complications such as violent reactions, psychosis, overdose and drug-dependency [76]. In terms of physiology, the anticholinergic properties of diphenhydramine has been shown to reduce the metabolism of methaqualone, thus increasing the hypnotic potency of methaqualone, an effect desired by drug users and abusers [76,67,77,78]. This is likely why the clandestine manufacturing of methaqualone with diphenhydramine has continued. The presence of methaqualone-diphenhydramine drug combination in this study may reflects the on-going availability from illegal manufacturing in and around the Cape Town area, which consequently suggests that economic-compulsive and/or systemic violence is associated with the use and abuse of methaqualone.

Poly-drug or poly-substance use, where two or more substances are used at the same time, is very common in South Africa. For instance, the "white pipe" in which cannabis is smoked with methaqualone. Mandrax and methamphetamine are both among the leading drugs of abuse in South Africa [68]. The use and abuse of methamphetamine, locally known as 'tik' is most common among the Coloured population of Cape Town in the Western Cape. It became the most widely used drug among poor communities because it is relatively cheap compared to other drugs. Tik use has now become a crucial heath issue in South Africa due to its association with gang-related crime, high-risk behaviour, aggression and violence [63,68,79]. In this study, a high frequency of diphenhydramine-methaqualone-methamphetamine drug combination was obtained in homicidal deaths, particularly among gunshot and stabbing cases. This finding supports the fact that both methaqualone (mandrax) and methamphetamine may both be crucial substances involved in drug markets related with gang warfare in Cape Town. The reasons for using multiple substances simultaneously remains unclear, however, one explanation for the combinations found is that smoking methaqualone may help drug users to sleep and relax after the "rush/high" experienced from using methamphetamine. For example, the effects of tik (crystal methamphetamine) including euphoria, increased CNS activity, anxiety, sweating, and palpitations are much stronger and last longer than the other forms of methamphetamine, however, the "crash" experienced is much worse [80]. Therefore, the sedating and hypnotic effects of methaqualone and diphenhydramine may be used to relieve some of the unpleasant side-effects of methamphetamine. This may be true for other sedating drugs such cannabis and benzodiazepines. Further studies are necessary to investigate the role and extent of poly-drug use, particularly the methaqualone-methamphetamine-diphenhydramine combination in South Africa in order to understand its association with fatal violence. This indicates the necessity for quantitative post-mortem toxicology in violent fatalities, so as to investigate the contributory nature of the combinations of these drugs in violent fatalities.

Limitations and Improvements

Like all research, this study has some limitations. Firstly, the study is preliminary in nature with a simple descriptive and qualitative design, therefore, no control group was used and no quantitative conclusions can be made on the results obtained as this requires a larger undertaking. Increasing the size and extent of the study would allow forensic researchers to make quantitative and comparative inferences for future use. For example, research in gender differences in drugs detected in violent deaths, toxicological comparisons between different types of unnatural deaths with the inclusion of control groups, evaluating drug combinations in violent fatalities in a South African context, and long-term studies looking at the trends and patterns in drug detected among deaths due to violence. This study has produced the preliminary data to indicate necessity of these further investigations. Secondly, due to the limited duration of the study, a relatively small sample size was used. This affected the ability to conduct certain statistical tests on the data collected. For instance, both SUI and ACC groups only had 5 cases each in total. Statistically accurate and representative information could not be drawn from the results, which was therefore described using relative proportions and percentages. This study did indicate the alarmingly high prevalence of homicides in comparison to other violent deaths within a time period in the West Metropole of Cape Town.

The authors investigated drugs other than alcohol as South African studies show there is a high prevalence of ethanol in violent fatalities [81], and hence the focus of this study on the presence of other drugs. However, the study has indicated that in future alcohol analyses should be performed in conjunction with analyses for drugs of abuse. Importantly, although the study examined the prevalence of substances that are present at the time of death, the results do not directly or indirectly correlate with the level of intoxication in the substance user at the time of the incident. The results obtained on drugs detected were pooled together from all samples (femoral blood, heart blood, urine, bile, and/or vitreous humor) analysed for each case, therefore the substance(s) detected in each specimen analysed may differ from each other (see Appendix A). Additionally, the proportions of substances present and drug users obtained in the study is likely to be an underestimate due to various circumstantial factors, such as the death of drug users who did not use drugs prior to death but died of a drug-related violent death.

It is important to mention that due to limited time and resources, no replication tests were conducted during the toxicological analysis, which are vital for confirmatory detection for future studies. The authors suggest to perform confirmatory analyses, preferably using a different analytical technique and instrument, and conducting quantitative analyses on interesting findings. In terms of specimen sampling, although blood analyses provide a measure of recent consumption, they do not provide any information on long term patterns. Although hair was collected in this study, due to limited analytical resources and scope of the project it was not analysed. Hair analysis would provide a larger period of time to look into the patterns of drug use and abuse over the time prior to the violent incident and hence should be considered for future research. In addition, due to time and cost considerations, the analytical component of this project is a preliminary screening based on the ABSciex iMethod (Part A, Appendix C) which screens against a database. In future, a method validation for criteria relevant to the research outcome should be conducted. Finally, post-mortem data is limited to victims at the Salt River mortuary and, as such, no inferences on the presence of drugs in perpetrators can be drawn as it was not possible to distinguish victims and perpetrators in the violent cases recorded.

Conclusions

This study provides the basis for future research into investigating post-mortem toxicology of violent fatalities in Cape Town, South Africa. Consistent with international research, psychoactive substances were present in the majority of violent deaths related to homicide, non-overdose suicide, and accidents. However, notable differences were observed in the toxicology of the different types of violent death in terms of victim demographics, circumstances of death, and suspected manner of death. The findings recognize the existence of a complex and dynamic relationship between substance use and deaths due to violent acts in a South African setting. While there are limitations to the interpretation and use of the data obtained in this study, the overall outcome has paved the way for future investigation to unravel some aspects of the interactions between drug use and violence-related mortality in South Africa. Further work, using toxicological data as a research tool, is essential to explore the extent and full nature of the identified relationship.

Key points

1. Drugs of abuse are prevalent in 60.0% of victims of violent deaths due to homicidal, suicidal, and accidental circumstances in this study.
2. More than one substance was detected in almost half of the victims of violent death.
3. Notable differences in toxicology findings were observed in terms of victim demographics, circumstances of death, manner of death, and drugs present.
4. The finding of methaqualone, methamphetamine, and diphenhydramine combinations is particularly common in violent fatalities in Cape Town, South Africa.
5. Toxicological analyses are important and useful research tools to investigate the involvement of drugs of abuse in violence-related mortality issues, and need to be utilised in further investigation of these larger issues.

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Compliance with Ethical Standards

The authors declare that they have no conflict of interest with the organization funding this research. The study was reviewed and accepted by the Human Research Ethics Committee of the University of Cape Town (HREC Ref 324/2015). All procedures performed were in accordance with the ethical standards of the UCT's HREC and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the next-of-kin of all individuals included in the study.

References

1. Athanasiadis L. Drugs, alcohol and violence. Curr. Opin. Psychiatry. 1999;12:281–6.

2. Boles SM, Miotto K. Substance abuse and violence: A review of the literature. Aggress. Violent Behav. 2003;8:155–74.

3. Parker RN, Auerhahn K. Alcohol, Drugs, and Violence. Annu. Rev. Sociol. 1998;24:291–311.

4. Johnson EM, Belfer ML. Substance abuse and violence: cause and consequence. J. Health Care Poor Underserved. 1995;6:113–21; discussion 121–3.

5. Haggård-Grann U, Hallqvist J, Långström N, Möller J. The role of alcohol and drugs in triggering criminal violence: a case-crossover study*. Addiction. England; 2006;101:100–8.

6. Shaw J, Hunt IM, Flynn S, Amos T, Meehan J, Robinson J, et al. The role of alcohol and drugs in homicides in England and Wales. Addiction. 2006;101:1117–24.

7. Weiner MD, Sussman S, Sun P, Dent C. Explaining the link between violence perpetration, victimization and drug use. Addict. Behav. 2005;30:1261–6.

8. Chermack ST, Bohnert ASB, Price AM, Austin K, Ilgen MA. Substance use disorders and homicide death in veterans. J. Stud. Alcohol Drugs. 2012;73:10–4.

9. Goldstein PJ. The Drugs/Violence Nexus: A Tripartite Conceptual Framework. J. Drug Issues. 1985;15:493–506.

10. Ousey GC, Lee MR. Examining the conditional nature of the illicit drug market-homicide relationship: a partial test of the theory of contingent causation. Criminology. 2002;40:73–102.

11. Tardiff K, Marzuk PM, Lowell K, Portera L, Leon AC. A study of drug abuse and other causes of homicide in New York. J. Crim. Justice. 2002;30:317–25.

12. Varano SP, McCluskey JD, Patchin JW, Bynum TS. Exploring the drugs-homicide connection. J. Contemp. Crim. Justice. 2004;20:369–92.

13. UNODC Global Study on Homicide 2013 (United Nations publication, Sales No. 14.IV.1).

14. Ellickson PL, Tucker JS, Klein DJ. Ten-year prospective study of public health problems associated with early drinking. Pediatrics. 2003;111:949–55.

15. Lennings CJ, Copeland J, Howard J. Substance use patterns of young offenders and violent crime. Aggress. Behav. 2003;29:414–22.

16. Scott KD, Schafer J, Greenfield TK. The role of alcohol in physical assault perpetration and victimization. J. Stud. Alcohol. 1999;60:528–36.

17. Wells S, Graham K. Aggression involving alcohol: relationship to drinking patterns and social context. Addiction. 2003;98:33–42.

18. Hoaken PNSS, Stewart SH. Drugs of abuse and the elicitation of human aggressive behavior. Addict. Behav. 2003;28:1533–54.

19. Ramstedt M. Population drinking and homicide in Australia: A time series analysis of the period 1950-2003. Drug Alcohol Rev. 2011;30:466–72.

20. Stickley A, Razvodovsky Y. The effects of beverage type on homicide rates in Russia, 1970-2005. Drug Alcohol

Rev. 2012;31:257–62.

21. Pridemore WA. Vodka and Violence: Alcohol Consumption and Homicide Rates in Russia. Am. J. Public Health. 2002;92:1921–30.

22. Wilcox HC, Conner KR, Caine ED. Association of alcohol and drug use disorders and completed suicide: an empirical review of cohort studies. Drug Alcohol Depend. 2004;76 Suppl:11–9.

23. Hulse GK, English DR, Milne E, Holman CD. The quantification of mortality resulting from the regular use of illicit opiates. Addiction. 1999;94:221–9.

24. Darke S, Degenhardt L, Mattick R. Mortality amongst Illicit Drug Users: Epidemiology, Causes and Intervention. Cambridge: Cambridge University Press; 2006.

25. Room R, Rossow I. The share of violence attributable to drinking. J. Subst. Use. 2001;6:218–28.

26. Mathews S, Abrahams N, Jewkes R, Martin LJ, Lombard C. Alcohol use and its role in female homicides in the Western Cape, South Africa. J. Stud. Alcohol Drugs. 2009;70:321–7.

27. Tardiff K, Marzuk PM, Leon AC, Hirsch CS, Stajić M, Portera L, et al. Cocaine, opiates, and ethanol in homicides in New York City: 1990 and 1991. J. Forensic Sci. 1995;40:387–90.

28. Garriott JC. Drug use among homicide victims. Changing patterns. Am. J. Forensic Med. Pathol. 1993;14:234–7.

29. Kuhns JB, Maguire ER. Drug and alcohol use by homicide victims in Trinidad and Tobago, 2001-2007. Forensic Sci. Med. Pathol. 2012;8:243–51.

30. Hedlund J, Ahlner J, Kristiansson M, Sturup J. A population-based study on toxicological findings in Swedish homicide victims and offenders from 2007 to 2009. Forensic Sci. Int. 2014;244:25–9.

31. Darke S, Duflou J. Toxicology and circumstances of death of homicide victims in New South Wales, Australia 1996-2005. J. Forensic Sci. 2008;53:447–51.

32. Tardiff AKJ, Wallace Z, Tracy M, Piper TM, Vlahov D, Galea S. Drug and alcohol use as determinants of New York City homicide trends from 1990 to 1998. J. Forensic Sci. 2005;50:470–4.

33. Darke S, Duflou J, Torok M. Drugs and violent death: Comparative toxicology of homicide and non-substance toxicity suicide victims. Addiction. 2009;104:1000–5.

34. Darke S. The toxicology of homicide offenders and victims: A review. Drug Alcohol Rev. 2010;29:202–15.

35. Ohberg A, Vuori E, Ojanperä I, Lonngvist J. Alcohol and drugs in suicides. Br. J. Psychiatry. 1996;169:75–80.

36. Rich CL, Dhossche DM, Ghani S, Isacsson G. Suicide methods and presence of intoxicating abusable substances: some clinical and public health implications. Ann. Clin. Psychiatry. 1998;10:169–75.

37. Coklo M, Stemberga V, Cuculic D, Sosa I, Bosnar A. Toxicology and methods of committing suicide other than overdose. Med. Hypotheses. 2009;73:809–10.

38. Marzuk PM, Tardiff K, Leon AC, Hirsch CS, Stajic M, Hartwell N, et al. Use of prescription psychotropic drugs among suicide victims in New York City. Am. J. Psychiatry. 1995;152:1520–2.

39. Dhossche DM, Rich CL, Ghani SO, Isacsson G. Patterns of psychoactive substance detection from routine toxicology of suicides in Mobile, Alabama, between 1990 and 1998. J. Affect. Disord. 2001;64:167–74.

40. Shields LBE, Hunsaker DM, Hunsaker JC, Ward MK. Toxicologic findings in suicide: a 10-year retrospective review of Kentucky medical examiner cases. Am. J. Forensic Med. Pathol. 2006;27:106–12.

41. Centers for Disease Control and Prevention (CDC). Toxicology testing and results for suicide victims--13 states, 2004. MMWR. Morb. Mortal. Wkly. Rep. 2006;55:1245–8.

42. Darke S, Duflou J, Torok M. Toxicology and circumstances of completed suicide by means other than overdose. J. Forensic Sci. 2009;54:490–4.

43. Galea S, Ahern J, Tardiff K, Leon AC, Vlahov D. Drugs and firearm deaths in New York City, 1990-1998. J. Urban Health. 2002;79:70–86.

44. Gill JR, Lenz K a, Amolat MJ. Gunshot fatalities in children and adolescents in New York City. J. Forensic Sci. 2003;48:832–5.

45. Dhossche DM. Toxicology of suicide: touchstone for suicide prevention? Med. Sci. Monit. 2003;9:SR9–19.

46. Reeves RR, Ladner ME. Antidepressant-induced suicidality: an update. CNS Neurosci. Ther. 2010;16:227–34.

47. Breiding MJ, Wiersema B. Variability of undetermined manner of death classification in the US. Inj. Prev. 2006;12 Suppl 2:ii49–54.

48. Chandran A, Hyder AA, Peek-Asa C. The Global Burden of Unintentional Injuries and an Agenda for Progress. Epidemiol. Rev. 2010;32:110–20.

49. Matzopoulos R. Violent Deaths in SA: The 2003 National Injury Mortality Surveillance System. SA Crime Q. 2005;29–36.

50. Matzopoulos R, Prinsloo M, Pillay-van Wyk V, Gwebushe N, Mathews S, Martin LJ, et al. Injury-related mortality in South Africa: a retrospective descriptive study of postmortem investigations. Bull. World Health Organ. 2015;93:303–13.

51. Thomson JDS. Coloured homicide trends in South Africa. South African Crime Q. 2004;9–14.

52. Parry CD, Plüddemann A, Louw A, Leggett T. The 3-metros study of drugs and crime in South Africa: findings and policy implications. Am. J. Drug Alcohol Abuse. 2004;30:167–85.

53. Kapp C. Crystal meth boom adds to South Africa's health challenges. Lancet (London, England). 2008;371:193–4.

54. Wechsberg WM, Luseno WK, Karg RS, Young S, Rodman N, Myers B, et al. Alcohol, cannabis, and methamphetamine use and other risk behaviours among Black and Coloured South African women: a small randomized trial in the Western Cape. Int. J. Drug Policy. 2008;19:130–9.

55. City of Cape Town (2014). State of Cape Town Report 2014.

56. District Health Plan 2007-2008. Metro District Health Services (MDHS), Cape Town.; [Accessed 2015 Dec 22]. Available from: https://www.capetown.gov.za/

57. Western Cape Department of Health. Forensic Pathology Service. [Accessed 2015 Apr 7]. Available from: www.westerncape.gov.za

58. Republic of South Africa. The Inquests Act (Act 58 of 1959) (and its amendments). Pretoria: Government Printer. 1959.

59. South African Medical Research Council. A profile of fatal injuries in South Africa: Nineth annual report (2007) of the National Injury Mortality Surveillance System. Pretoria, South Africa; 2008.

60. South African Medical Research Council. A profile of fatal injuries in South Africa: Tenth annual report (2008) of the National Injury Mortality Surveillance System. Pretoria, South Africa; 2010.

61. Dresen S, Ferreirós N, Gnann H, Zimmermann R, Weinmann W. Detection and identification of 700 drugs by multi-target screening with a 3200 Q TRAP LC-MS/MS system and library searching. Anal. Bioanal. Chem. 2010;396:2425–34.

62. StataCorp. Stata Statistical Software: Release 14. College Station: TX: StataCorp LP; 2015.

63. Dada S, Town C, Burnhams NH, Town C, Williams Y, Town C, et al. Monitoring Alcohol and Drug Abuse Treatment Admissions in South Africa. Cape Town, South Africa; 2013.

64. Sheehan CM, Rogers RG, Williams GW, Boardman JD. Gender differences in the presence of drugs in violent deaths. Addiction. 2013;108:547–55.

65. Peden M, Van Der Spuy J, Smith P, Bautz P. Substance abuse and trauma in Cape Town. South African Med. J. 2000;90:251–5.

66. da Silva AA, Bester MJ, Pretorius E. Effects of Mandrax and Cannabis on the cellular function of chick embryonic neurons. Environ. Toxicol. Pharmacol. 2007;23:82–8.

67. Williams ME, Davis SS, Poxon R, Kendall MJ, Mitchard M. The influence of diphenhydramine on the absorption of methaqualone in man. Br. J. Clin. Pharmacol. 1974;1:259–64.

68. Peltzer K, Ramlagan S, Johnson BD, Phaswana-Mafuya N. Illicit drug use and treatment in South Africa: A review. Subst. use misuse. 2010;45:2221–43.

69. van Heerden MS, Grimsrud AT, Seedat S, Myer L, Williams DR, Stein DJ. Patterns of substance use in South Africa: results from the South African Stress and Health study. South African Med. J. 2009;99:358–66.

70. Darke S, Kaye S, McKetin R, Duflou J. Major physical and psychological harms of methamphetamine use. Drug Alcohol Rev. 2008;27:253–62.

71. Garlow SJ. Age, gender, and ethnicity differences in patterns of cocaine and ethanol use preceding suicide. Am. J. Psychiatry. 2002;159:615–9.

72. Kuhns JB, Wilson DB, Maguire ER, Ainsworth SA, Clodfelter TA. A meta-analysis of marijuana, cocaine and opiate toxicology study findings among homicide victims. Addiction. 2009;104:1122–31.

73. Moore TM, Stuart GL. A review of the literature on marijuana and interpersonal violence. Aggress. Violent Behav. 2005;10:171–92.

74. United Nations Office on Drugs and Crime. World Drug Report 2014 (United Nations publication, Sales No. E.14.XI.7).

75. Parry CDH, Plüddemann A, Donson H, Sukhai A, Marais S, Lombard C. Cannabis and other drug use among trauma patients in three South African cities, 1999-2001. South African Med. J. 2005;95:429–32.

76. Tennant FS. Complications of methaqualone-diphenhydramine (Mandrax R) abuse. Br. J. Addict. Alcohol Other Drugs. 1973;68:327–30.

77. Hindmarsh KW, Wallace SM, Schneider CB, Korchinski ED. Methaqualone-diphenhydramine interaction study in humans. J. Pharm. Sci. 1983;72:176–80.

78. Hindmarsh KW, Hamon NW, LeGatt DF, Wallace SM. Effect of diphenhydramine on methaqualone metabolism: an in vitro study. J. Pharm. Sci. 1978;67:1547–50.

79. Chermack ST, Blow FC. Violence among individuals in substance abuse treatment: the role of alcohol and cocaine consumption. Drug Alcohol Depend. 2002;66:29–37.

80. Edwards GI. Drugs on your streets (illustrated). The Rosen Publishing Group; 1997.

81. Rataemane S, Rataemane L. Alcohol in South Africa. Int. J. Drug Policy. 2006;17:373–5.

Part D

Appendices

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Blenkinsopp A, Paxton P. Symptoms in the pharmacy: a guide to the management of common illness. 3rd ed. Oxford: Blackwell Science; 1998.

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3. *Technical Appendices*

Appendix A

Table 1 Data recorded for 104 cases due to violent death from the Salt River mortuary, Cape Town, Aug-Nov 2015.

ID	Age (yrs)	Sex	Ethnicity	Substances Present	Suspected Manner of Death	Apparent Cause of death	Drug(s) present in specimens collected for toxicology analysis				
							Femoral Blood	Heart Blood	Urine	Vitreous Humor	Bile
1	26	M	C	Yes	HOM	Shot	Diazepam	Nordiazepam	-	-	N/A
2	33	M	C	Yes	HOM	Shot	-	N/A	Methamphetamine, Amphetamine	-	-
3	46	M	C	No	HOM	Shot	-	-	-	-	N/A
4	25	M	A	Yes	HOM	Stab	-	N/A	Diphenhydramine, Methamphetamine, Amphetamine	-	THC-COOH
5	42	M	A	Yes	HOM	Shot	-	N/A	Amphetamine	-	-
6	19	M	A	No	HOM	Stab	-	-	-	-	N/A
7	59	M	A	No	HOM	Shot	-	N/A	N/A	-	-
8	36	F	A	No	HOM	Shot	-	-	N/A	-	-
9	28	M	A	Yes	HOM	Shot	-	N/A	Atropine, Benzoylecgonine, Cocaine	-	Benzoylecgonine
10	44	M	C	No	HOM	Assault	-	-	-	-	N/A
11	29	M	A	No	HOM	Shot	-	-	-	-	-
12	75	F	W	Yes	SUI	Hang	-	-	Desipramine	-	N/A
13	30	M	A	No	HOM	Stab	-	-	-	-	N/A
14	28	M	A	Yes	HOM	Assault	Diphenhydramine, Methaqualone	Diphenhydramine, Methaqualone	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone	N/A
15	20	M	C	Yes	HOM	Shot	Methamphetamine, Diphenhydramine, Methaqualone	Diphenhydramine, Methaqualone	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	Methaqualone	N/A
16	25	M	A	No	HOM	Stab	-	-	-	-	N/A
17	50	M	C	Yes	HOM	Stab	Diphenhydramine, Methaqualone	Diphenhydramine, Methaqualone	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone, Morphine, Hydromorphone	Diphenhydramine, Methaqualone	N/A
18	28	M	A	No	HOM	Shot	-	-	-	-	N/A
19	38	M	A	No	HOM	Shot/Stab	-	-	-	-	N/A
20	38	M	A	No	HOM	Stab	-	N/A	-	-	-
21	26	M	C	Yes	HOM	Shot	Methamphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone, Morphine-3-B-D-glucuronide	-	N/A
22	31	M	A	Yes	HOM	Stab	-	-	-	Sibutramine	N/A
23	17	M	A	Yes	HOM	Assault	Methamphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone, Morphine-3-B-D-glucuronide	-	N/A
24	49	F	A	Yes	HOM	Shot	Metformin	Metformin, Amlodipine	N/A	-	Metformin, Amlodipine
25	25	M	C	Yes	HOM	Stab	-	-	N/A	-	Acetaminophen
26	10	M	A	Yes	ACC	Drowning	-	-	Codeine	-	N/A
27	21	M	A	No	HOM	Shot	-	N/A	N/A	-	-
28	26	M	A	No	HOM	Shot	-	-	-	-	N/A
29	20	M	A	No	HOM	Stab	-	-	-	-	N/A
30	32	M	C	Yes	HOM	Shot	Methamphetamine	Methamphetamine	Methamphetamine, Amphetamine, Diphenhydramine	-	Methamphetamine, Diphenhydramine
31	42	M	A	No	SUI	Hang	-	-	-	-	N/A
32	20	M	A	Yes	HOM	Assault	-	-	Lidocaine	-	N/A
33	20	M	A	Yes	HOM	Stab	Ketamine	Ketamine	Ketamine	Ketamine	N/A
34	25	M	A	No	HOM	Assault	-	-	-	-	N/A

35	25	M	A	No	HOM	Stab	-	N/A	-	-	-
36	28	M	C	Yes	HOM	Shot	Methaqualone	Methaqualone	Methaqualone, THC-COOH, Diphenhydramine	Methaqualone	-
37	60	M	W	No	HOM	Shot	-	-	-	-	-
38	50	M	W	No	HOM	Shot	-	-	-	-	-
39	49	M	C	No	HOM	Shot	-	-	-	-	N/A
40	18	M	C	Yes	HOM	Shot	Diphenhydramine, Methaqualone, Atenolol	Diphenhydramine, Methaqualone	Methaqualone, Clomipramine	Diphenhydramine	N/A
41	20	M	A	Yes	HOM	Stab	THC-OH	THC-OH, Lidocaine, Norfluoxetine	-	-	N/A
42	20	M	C	Yes	HOM	Shot	THC-OH, Methaqualone, Norfluoxetine	THC-OH, Methaqualone, Norfluoxetine	Nordiazepam, Morphine-3-B-D-glucuronide	Methaqualone	N/A
43	27	M	A	Yes	HOM	Assault	N/A	N/A	MDMA	-	Diphenhydramine Methamphetamine, THC-OH, Temazepam, Diltiazem
44	30	M	A	Yes	HOM	Assault	Diphenhydramine, THC-OH, Methaqualone, Norfluoxetine	THC-OH, Methaqualone	Methaqualone, Lidocaine	Methaqualone	N/A
45	63	F	W	Yes	ACC	Drowning	THC-OH, Fluoxetine, Protriptyline, Nortriptyline	N/A	N/A	Norfluoxetine, Protriptyline, Nortriptyline, Tramadol	Nordiazepam, THC-OH, Diltiazem, Protriptyline, Nortriptyline, Tramadol, Fentanyl, Dexamethasone
46	26	M	C	Yes	HOM	Shot	Diphenhydramine, THC-OH, Methaqualone	Diphenhydramine, THC-OH, Methaqualone	Diphenhydramine, Ranitine, Reserpine	-	THC-OH, Nordiazepam
47	35	M	A	Yes	HOM	Stab	THC-OH	THC-OH	-	-	N/A
48	30	M	C	Yes	HOM	Shot	THC-OH, Methaqualone	Diphenhydramine, THC-OH, Methaqualone	Diphenhydramine	Methamphetamine, Methaqualone	N/A
49	30	M	A	Yes	HOM	Stab	Lidocaine	THC-OH, Lidocaine, Ketamine, Norfentanyl	Lidocaine	THC-OH, Temazepam, Diltiazem	N/A
50	21	M	A	Yes	HOM	Assault	THC-OH, Methaqualone	THC-OH, Diphenhydramine	MDMA	Diphenhydramine	N/A
51	30	M	A	No	HOM	Stab	-	-	-	-	N/A
52	21	M	A	No	HOM	Shot	-	-	-	-	N/A
53	23	M	A	Yes	HOM	Shot	Methaqualone	-	N/A	-	-
54	32	M	C	Yes	HOM	Shot	-	N/A	Diphenhydramine	Diphenhydramine	Diphenhydramine, THC-COOH
55	30	M	A	No	HOM	Stab	-	-	-	-	N/A
56	28	M	A	Yes	HOM	Shot	-	-	Amphetamine, Codeine	-	N/A
57	23	M	A	No	HOM	Shot	-	-	N/A	-	-
58	24	M	A	No	HOM	Assault	-	-	-	-	N/A
59	25	M	C	Yes	HOM	Shot	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	N/A	Diazepam, Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone, Morphine-3-B-D-glucuronide	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	Diazepam, Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone, Morphine-3-B-D-glucuronide
60	41	M	A	Yes	HOM	Shot	-	N/A	Amphetamine, Codeine	-	N/A
61	35	M	A	No	ACC	Freak	-	-	-	-	N/A
62	17	M	A	Yes	HOM	Stab	N/A	-	Codeine, Phencyclidine, Methcathinone	-	Methcathinone
63	31	M	A	Yes	HOM	Shot	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	N/A	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone
64	65	M	C	Yes	SUI	Hang	Metformin	Metformin	N/A	Metformin	Metformin, Amlodipine
65	16	M	A	Yes	HOM	Shot	Diphenhydramine, Methaqualone, Salicylamide	Diphenhydramine, Methaqualone	Diphenhydramine, Methaqualone, Morphine-3-B-D-glucuronide	Methamphetamine, Diphenhydramine, Methaqualone	N/A
66	15	F	W	Yes	SUI	Hang	Acetaminophen, Codeine	Diphenhydramine, Acetaminophen, Codeine	N/A	Acetaminophen, Codeine, Doxylamine	Acetaminophen, Codeine
67	34	M	C	Yes	HOM	Shot	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone, Morphine, Morphine-3-B-D-glucuronide	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	N/A
68	32	M	A	No	HOM	Stab	-	-	-	-	N/A
69	20	M	A	No	HOM	Shot	-	-	-	-	-

70	21	M	A	Yes	HOM	Assault	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Amphetamine, Diphenhydramine, Cocaine, Methaqualone, Morphine-3-B-D-glucuronide	Methamphetamine, Amphetamine, Diphenhydramine, Methaqualone, Ranitine	N/A
71	23	M	A	No	HOM	Shot	-	-	-	-	N/A
72	20	M	A	Yes	HOM	Assault	Methamphetamine, Diphenhydramine, Methaqualone	Diphenhydramine, Methaqualone	Methamphetamine, Diphenhydramine,	Methamphetamine, Diphenhydramine, Methaqualone	N/A
73	18	M	A	Yes	HOM	Assault	Methaqualone	Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone
74	30	M	A	Yes	HOM	Assault	Methaqualone	Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone	Diphenhydramine, Methaqualone	N/A
75	32	M	A	Yes	HOM	Stab	-	-	Methamphetamine, Diphenhydramine	-	N/A
76	26	M	A	Yes	HOM	Shot	-	Midazolam	Lidocaine	Lidocaine	N/A
77	21	M	A	Yes	HOM	Stab	Methaqualone	Methaqualone	Methamphetamine, Diphenhydramine	-	-
78	31	M	A	No	HOM	Stab	-	-	-	-	N/A
79	31	M	C	Yes	HOM	Stab	-	N/A	Methamphetamine	Methamphetamine	-
80	15	M	C	Yes	HOM	Shot	Methaqualone	Methaqualone	Diphenhydramine	Diphenhydramine, Methaqualone	N/A
81	23	M	C	Yes	HOM	Assault	Methaqualone	Methaqualone	-	-	-
82	25	M	C	No	HOM	Assault	-	-	-	-	N/A
83	31	M	A	No	HOM	Shot	-	-	-	-	-
84	26	M	C	Yes	HOM	Shot	Methaqualone	Diphenhydramine, Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone	Methaqualone	N/A
85	33	M	A	No	HOM	Stab	-	-	-	-	N/A
86	25	M	C	Yes	HOM	Shot	Methaqualone	Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone	-	N/A
87	41	M	C	No	ACC	Drowning	-	-	-	-	-
88	28	M	C	Yes	HOM	Shot	-	-	Lidocaine	-	N/A
89	33	M	A	No	HOM	Stab	-	-	-	-	N/A
90	26	M	A	Yes	HOM	Assault	-	-	Lidocaine, Ketamine	Lidocaine, Ketamine	N/A
91	35	M	A	Yes	HOM	Stab	Methaqualone	Methaqualone	Diphenhydramine, Methaqualone	THC-OH, Methaqualone	N/A
92	40	M	A	No	HOM	Assault	-	-	-	-	-
93	30	M	A	No	ACC	Electrocutio n	-	-	-	-	N/A
94	24	M	A	No	HOM	Stab	-	-	-	-	N/A
95	18	M	A	Yes	HOM	Shot	Methaqualone	Diphenhydramine, Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone	-	N/A
96	32	M	A	No	HOM	Shot	-	-	-	-	N/A
97	27	M	A	No	SUI	Hang	-	-	-	-	N/A
98	23	M	C	Yes	HOM	Shot	Diphenhydramine, Methaqualone	Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone	Diphenhydramine, THC-OH, Methaqualone	N/A
99	58	M	A	No	HOM	Shot	-	-	-	-	N/A
10 0	37	M	C	Yes	HOM	Stab	-	Diphenhydramine, Methaqualone	Methamphetamine, Diphenhydramine, Methaqualone	-	N/A
10 1	52	M	C	Yes	HOM	Shot	Diphenhydramine, Methaqualone	Diphenhydramine, Methaqualone	Diphenhydramine, Methaqualone	Methaqualone	N/A
10 2	25	M	A	Yes	HOM	Assault/Stab	-	Methamphetamine, Methaqualone	Methamphetamine	-	N/A
10 3	30	M	A	Yes	HOM	Assault	N/A	-	Methamphetamine, Diphenhydramine	-	Diphenhydramine
10 4	32	M	A	Yes	HOM	Stab		Ketamine	Ketamine	Ketamine	N/A

M, Male; F, Female; A, Black African; C, Coloured; W, White; HOM, Homicide; SUI, Suicide; ACC, Accident; N/A, Not available.

4. List of Literature Reviewed

1. World Health Organization (WHO). Global status report on violence prevention 2014. [Accessed 2016 Jan 10]. Available from: http://www.who.int/violence_injury_prevention/

2. World Health Organization (WHO). Injuries and Violence: The Facts 2010. Geneva, Switzerland.; [Accessed 2016 Jan 5]. Available from: http://www.who.int/violence/

3. Dada MA, McQuoid-Mason DJ. Introduction to Medico-legal Practice. Durban: Butterworths; 2001.

4. World Health Organization (WHO). Global Health Observatory Data Repository: Cause of death 2008 summary tables. 2008 [Accessed 2015 Apr 7]. Available from: www.who.int

5. Bartolomeos K, Kipsaina C, Grills N, Ozanne-Smith J, Peden M (eds). Fatal injury surveillance in mortuaries and hospitals: a manual for practitioners. Geneva: World Health Organization; 2012.

6. Krug EG, Dahlberg LL, Mercy JA, Zwi AB, Lozano R (eds). World report on violence and health. Geneva; 2002.

7. Bradshaw D, Nanna N, Laubscher R, Groenewald P, Joubert J, Nojilana B, et al. South African National Burden of Disease Study 2000: Estimates of Provincial Mortality. Cape Town; 2004.

8. Matzopoulos R, Norman R, Bradshaw D. The burden of injury in South Africa: Fatal injury trends and international comparisons. In: Suffla S, Van Niekerk A, Duncan N, editors. Crime, Violence Inj. Prev. South Africa Dev. Challenges. Medical Research Council- University of South Africa; 2004. p. 9–21.

9. Seedat M, Van Niekerk A, Jewkes R, Suffla S, Ratele K. Violence and injuries in South Africa: prioritising an agenda for prevention. Lancet (London, England). 2009;374:1011–22.

10. South African Medical Research Council. A profile of fatal injuries in South Africa: Tenth annual report (2008) of the National Injury Mortality Surveillance System. Pretoria, South Africa; 2010.

11. South African Medical Research Council. A profile of fatal injuries in South Africa: Nineth annual report (2007) of the National Injury Mortality Surveillance System. Pretoria, South Africa; 2008.

12. Groenewald P, Berteler M, Bradshaw D, Coetzee D, Cornelius K, Daniels J, et al. Western Cape Mortality Profile 2010. Cape Town: Cape T. South African Med. Res. Counc. 2013. Cape Town; 2013.

13. United Nations Office on Drugs and Crime. World Drug Report 2014 (United Nations publication, Sales No. E.14.XI.7).

14. UNODC. World Drug Report 2011 (United Nations Publication, Sales No. E.11.XI.10).

15. Dada S, Town C, Burnhams NH, Town C, Williams Y, Town C, et al. Monitoring Alcohol and Drug Abuse Treatment Admissions in South Africa. Cape Town, South Africa; 2013.

16. Wechsberg WM, Luseno WK, Karg RS, Young S, Rodman N, Myers B, et al. Alcohol, cannabis, and methamphetamine use and other risk behaviours among Black and Coloured South African women: a small randomized trial in the Western Cape. Int. J. Drug Policy. 2008;19:130–9.

17. City of Cape Town (2014). State of Cape Town Report 2014.

18. Kapp C. Crystal meth boom adds to South Africa’s health challenges. Lancet (London, England). 2008;371:193–4.

19. Boles SM, Miotto K. Substance abuse and violence: A review of the literature. Aggress. Violent Behav. 2003;8:155–74.

20. Parker RN, Auerhahn K. Alcohol, Drugs, and Violence. Annu. Rev. Sociol. 1998;24:291–311.

21. Darke S, Duflou J. Toxicology and circumstances of death of homicide victims in New South Wales, Australia 1996-2005. J. Forensic Sci. 2008;53:447–51.

22. Darke S, Duflou J, Torok M. Drugs and violent death: Comparative toxicology of homicide and non-substance toxicity suicide victims. Addiction. 2009;104:1000–5.

23. Room R, Rossow I. The share of violence attributable to drinking. J. Subst. Use. 2001;6:218–28.

24. Darke S, Degenhardt L, Mattick R. Mortality amongst Illicit Drug Users: Epidemiology, Causes and Intervention. Cambridge: Cambridge University Press; 2006.

25. UNODC Global Study on Homicide 2013 (United Nations publication, Sales No. 14.IV.1).

26. Galea S, Ahern J, Tardiff K, Leon AC, Vlahov D. Drugs and firearm deaths in New York City, 1990-1998. J. Urban Health. 2002;79:70–86.

27. Gill JR, Catanese C. Sharp injury fatalities in New York City. J. Forensic Sci. 2002;47:554–7.

28. Gill JR, Lenz K a, Amolat MJ. Gunshot fatalities in children and adolescents in New York City. J. Forensic Sci. 2003;48:832–5.

29. Tardiff K, Marzuk PM, Leon AC, Hirsch CS, Stajić M, Portera L, et al. Cocaine, opiates, and ethanol in homicides in New York City: 1990 and 1991. J. Forensic Sci. 1995;40:387–90.

30. Tardiff AKJ, Wallace Z, Tracy M, Piper TM, Vlahov D, Galea S. Drug and alcohol use as determinants of New York City homicide trends from 1990 to 1998. J. Forensic Sci. 2005;50:470–4.

31. Hedlund J, Ahlner J, Kristiansson M, Sturup J. A population-based study on toxicological findings in Swedish homicide victims and offenders from 2007 to 2009. Forensic Sci. Int. 2014;244:25–9.

32. Harris EC, Barraclough B. Suicide as an outcome for mental disorders. A meta-analysis. Br. J. Psychiatry. 1997;170:205–28.

33. Darke S, Duflou J, Torok M. Toxicology and circumstances of completed suicide by means other than overdose. J. Forensic Sci. 2009;54:490–4.

34. Wilcox HC, Conner KR, Caine ED. Association of alcohol and drug use disorders and completed suicide: an empirical review of cohort studies. Drug Alcohol Depend. 2004;76 Suppl:11–9.

35. Centers for Disease Control and Prevention (CDC). Toxicology testing and results for suicide victims--13 states, 2004. MMWR. Morb. Mortal. Wkly. Rep. 2006;55:1245–8.

36. Crombie IK, Pounder DJ, Dick PH. Who takes alcohol prior to suicide? J. Clin. Forensic Med. 1998;5:65–8.

37. Dhossche DM, Rich CL, Ghani SO, Isacsson G. Patterns of psychoactive substance detection from routine toxicology of suicides in Mobile, Alabama, between 1990 and 1998. J. Affect. Disord. 2001;64:167–74.

38. Marzuk PM, Tardiff K, Leon AC, Hirsch CS, Stajic M, Hartwell N, et al. Use of prescription psychotropic drugs among suicide victims in New York City. Am. J. Psychiatry. 1995;152:1520–2.

39. Shields LBE, Hunsaker DM, Hunsaker JC, Ward MK. Toxicologic findings in suicide: a 10-year retrospective review of Kentucky medical examiner cases. Am. J. Forensic Med. Pathol. 2006;27:106–12.

40. Rich CL, Dhossche DM, Ghani S, Isacsson G. Suicide methods and presence of intoxicating abusable substances: some clinical and public health implications. Ann. Clin. Psychiatry. 1998;10:169–75.

41. Ohberg A, Vuori E, Ojanperä I, Lonngvist J. Alcohol and drugs in suicides. Br. J. Psychiatry. 1996;169:75–80.

42. Coklo M, Stemberga V, Cuculic D, Sosa I, Bosnar A. Toxicology and methods of committing suicide other than overdose. Med. Hypotheses. 2009;73:809–10.

43. Mathers C, Fat DM, Boerma JT. The global burden of disease: 2004 update. Geneva, Switzerland: World Health Organization (WHO); 2008 [Accessed 2015 Nov 2]. Available from: www.who.int/evidence/bod

44. Warner M, Chen LH, Makuc DM, Anderson RN, Miniño AM. Drug poisoning deaths in the United States, 1980-2008. NCHS Data Brief. 2011;1–8.

45. Breiding MJ, Wiersema B. Variability of undetermined manner of death classification in the US. Inj. Prev. 2006;12 Suppl 2:ii49–54.

46. Donaldson AE, Larsen GY, Fullerton-Gleason L, Olson LM. Classifying undetermined poisoning deaths. Inj. Prev. 2006;12:338–43.

47. Western Cape Department of Health. Forensic Pathology Service. [Accessed 2015 Apr 7]. Available from: www.westerncape.gov.za

48. Parry CD, Plüddemann A, Louw A, Leggett T. The 3-metros study of drugs and crime in South Africa: findings and policy implications. Am. J. Drug Alcohol Abuse. 2004;30:167–85.

49. Thomson JDS. Coloured homicide trends in South Africa. South African Crime Q. 2004;9–14.

50. Peden M, Van Der Spuy J, Smith P, Bautz P. Substance abuse and trauma in Cape Town. South African Med. J. 2000;90:251–5.

51. Lennings CJ, Copeland J, Howard J. Substance use patterns of young offenders and violent crime. Aggress. Behav. 2003;29:414–22.

52. Friedman AS. Substance use/abuse as a predictor to illegal and violent behavior. Aggress. Violent Behav. 1998;3:339–55.

53. Wagner EF. Substance use and violent behavior in adolescence. Aggress. Violent Behav.

1996;1:375–87.

54. Moffat AC, Osselton D, Widdop B, Clarke EGC. Clarke's Analysis of Drugs and Poisons: In Pharmaceuticals, Body Fluids and Postmortem Material. 3rd ed. Pharmaceutical Press; 2004.

55. Tytgat J. Screening of Urine and Blood Using Limited Sample Preparation and Information Dependent Acquisition with LC-MS/MS as Alternative for Immunoassays in Forensic Toxicology. J. Forensic Toxicol. Pharmacol. 2013;02.

56. Deventer K, Pozo OJ, Verstraete AG, Van Eenoo P. Dilute-and-shoot-liquid chromatography-mass spectrometry for urine analysis in doping control and analytical toxicology. TrAC Trends Anal. Chem. 2014;55:1–13.

57. Goldstein PJ. The Drugs/Violence Nexus: A Tripartite Conceptual Framework. J. Drug Issues. 1985;15:493–506.

58. Nayar S, Pless IB. Injury Control - A Global View. Inj. Prev. 1997;3:231–2.

59. District Health Plan 2007-2008. Metro District Health Services (MDHS), Cape Town. [Accessed 2015 Dec 22]. Available from: https://www.capetown.gov.za/en/IDP/Statutory comp plans 201011/Annexure_F3_DHP_20072008_v4_Final_151206.pdf

60. Lehola P. How the Count was Done. Statistics South Africa (Statssa). 2012 [Accessed 2016 Jan 8]. p. 1–66. Available from: www.statssa.gov.za

61. Strategic Development Information and GID department. City of Cape Town – 2011 Census. City of Cape Town; 2012 [Accesseed 2015 Dec 20]. p. 1–5. Available from: https://www.capetown.gov.za/en/stats/Pages/Census2011.aspx

62. Republic of South Africa. The Inquests Act (Act 58 of 1959) (and its amendments). Pretoria: Government Printer.; 1959.

63. Republic of South Africa. National Health Act (Act No. 61 of 2003). Pretoria: Government Printer; 2003.

64. South African Police Services (SAPS). An analysis of the national crime statistics 2013/14. [Accessed 2016 Feb 4]. Available from: www.saps.gov.za

65. World Health Organization (WHO). Preventing suicide: A global imperative. Geneva; 2014 [Accessed 2015 Oct 5]. Available from: http://apps.who.int/

66. Bertolote J, Fleischmann A. A global perspective in the epidemiology of suicide. Suicidologi. 2002;7:6–8.

67. World Health Organization (WHO). Public health action for the prevention of suicide. 2012 [Accessed 2015 Dec 3]. p. 1–26. Available from: http://www.who.int/mental_health

68. Schlebusch L. Suicide prevention: a proposed national strategy for South Africa. Afr. J. Psychiatry. 2012;15:436–40.

69. Schlebusch L. Current perspectives on suicidal behaviour in South Africa. In: Suffla S, Van Niekerk A, Duncan D, editors. Crime, Violence Inj. Prev. South Africa Dev. Challenges. Tygerberg: Medical Research Council - UNISA Crime, Violence and Injury Lead Programme; 2004. p. 9–21.

70. Bantjes J, Kagee A. Epidemiology of suicide in South Africa: Setting an agenda for future research. South African J. Psychol. 2013;43:238–51.

71. Matzopoulos R, Prinsloo M, Pillay-van Wyk V, Gwebushe N, Mathews S, Martin LJ, et al. Injury-related mortality in South Africa: a retrospective descriptive study of postmortem investigations. Bull. World Health Organ. 2015;93:303–13.

72. Chandran A, Hyder AA, Peek-Asa C. The Global Burden of Unintentional Injuries and an Agenda for Progress. Epidemiol. Rev. 2010;32:110–20.

73. Norton R, Hyder A a, Bishai D, Peden M. Unintentional Injuries. Dis. Control Priorities Dev. Ctries. 2nd ed. World Bank Publications; 2006. p. 737–53.

74. World Health Organization (WHO). Management of substance abuse. Psychoactive substances. [Internet]. [cited 2016 Jan 26]. Available from: www.who.int

75. World Health Organization. Neuroscience of psychoactive substance use and dependence. Addiction. 2004;99:1361–2.

76. United Nations Office on Drugs and Crime. World Drug Report 2015 (United Nations publication, Sales No. E.15.XI.6).

77. Parry CDH. Substance Abuse in South Africa : Country Report Focussing On Young Persons. Tygerberg, Cape Town : Medical Research Council (MRC); 1998.

78. Plüddemann A, Dada S, Bhana A, Bachoo S, Perreira T, Freytag K, et al. Monitoring Alcohol & Drug Abuse Trends In South Africa (July 1996-June 2010). SACENDU Reasearch Br. 2010;13.

79. van Heerden MS, Grimsrud AT, Seedat S, Myer L, Williams DR, Stein DJ. Patterns of substance use in South Africa: results from the South African Stress and Health study. South African Med. J. 2009;99:358–66.

80. UNODC. World Drug Report 2010 (United Nations Publication, Sales No. E.10.XI.13).

81. Pluddemann A, Parry C, Fourie D, Dada S, Bhana A. Alcohol and Drug Abuse Trends: July - December 2009 (Phase 27). SACENDU Reasearch Br. 2010 [Accessed 2015 Nov 27]. Available from: http://www.hsrc.ac.za/en/research-data/view/5191

82. Lavine R. Psychopharmacological treatment of aggression and violence in the substance using population. J. Psychoactive Drugs. 1997;29:321–9.

83. Chermack ST, Blow FC. Violence among individuals in substance abuse treatment: the role of alcohol and cocaine consumption. Drug Alcohol Depend. 2002;66:29–37.

84. Hoaken PNSS, Stewart SH. Drugs of abuse and the elicitation of human aggressive behavior. Addict. Behav. 2003;28:1533–54.

85. Darke S, Torok M, Kaye S, Ross J, McKetin R. Comparative rates of violent crime among regular methamphetamine and opioid users: offending and victimization. Addiction. 2010;105:916–9.

86. Garlow SJ. Age, gender, and ethnicity differences in patterns of cocaine and ethanol use preceding suicide. Am. J. Psychiatry. 2002;159:615–9.

87. Kuhns JB, Wilson DB, Maguire ER, Ainsworth SA, Clodfelter TA. A meta-analysis of marijuana, cocaine and opiate toxicology study findings among homicide victims. Addiction. 2009;104:1122–31.

88. Marzuk PM, Tardiff K, Leon AC, Stajic M, Morgan EB, Mann JJ. Prevalence of cocaine use among residents of New York City who committed suicide during a one-year period. Am. J.

Psychiatry. 1992;149:371–5.

89. Johnson EM, Belfer ML. Substance abuse and violence: cause and consequence. J. Health Care Poor Underserved. 1995;6:113–21; discussion 121–3.

90. Roth JA. Psychoactive Substances and Violence. Washington, DC: National Institute of Justice, Office of Justice Programs; 1994.

91. Ryan T. Drugs, violence and governability in the future South Africa. Inst. Secur. Stud. Pap. 1997.

92. Copes H, Hochstetler A, Sandberg S. Using a Narrative Framework to Understand the Drugs and Violence Nexus. Crim. Justice Rev. 2015;40:32–46.

93. Oser CB, Mooney JL, Staton-Tindall M, Leukefeld CG. The drugs-violence nexus among rural felony probationers. J. Interpers. Violence. 2009;24:1285–303.

94. Bornman E, Van Eeden R, Wentzel M. Group membership as determinant of violence and conflict: The case of South Africa. Violence South Africa a Var. Perspect. HSRC Press; 1998. p. 85–116.

95. Rivara FP, Mueller BA, Somes G, Mendoza CT, Rushforth NB, Kellermann AL. Alcohol and illicit drug abuse and the risk of violent death in the home. JAMA. 1997;278:569–75.

96. Dhossche DM. Toxicology of suicide: touchstone for suicide prevention? Med. Sci. Monit. 2003;9:SR9–19.

97. Chermack ST, Bohnert ASB, Price AM, Austin K, Ilgen MA. Substance use disorders and homicide death in veterans. J. Stud. Alcohol Drugs. 2012;73:10–4.

98. Kuhns JB, Maguire ER. Drug and alcohol use by homicide victims in Trinidad and Tobago, 2001-2007. Forensic Sci. Med. Pathol. 2012;8:243–51.

99. Garriott JC. Drug use among homicide victims. Changing patterns. Am. J. Forensic Med. Pathol. 1993;14:234–7.

100. Darke S. The toxicology of homicide offenders and victims: A review. Drug Alcohol Rev. 2010;29:202–15.

101. Shaw J, Hunt IM, Flynn S, Amos T, Meehan J, Robinson J, et al. The role of alcohol and drugs in homicides in England and Wales. Addiction. 2006;101:1117–24.

102. Abel EL. Drugs and homicide in Erie County, New York. Int. J. Addict. 1987;22:195–200.

103. Adeagbo BA, Clark C, Collins KA. Homicides committed by youth assailants: a retrospective study. Am. J. Forensic Med. Pathol. 2008;29:219–23.

104. Goldstein PJ, Brownstein HH, Ryna PJ, Bellucci PA. Crack and homicide in New York City, 1988 a conceptually based event analysis. Contemp. Drug Probl. 1989;16:651–88.

105. Harruff RC, Francisco JT, Elkins SK, Phillips AM, Fernandez GS. Cocaine and homicide in Memphis and Shelby county: an epidemic of violence. J. Forensic Sci. 1988;33:1231–7.

106. Hood I, Ryan D, Monforte J, Valentour J. Cocaine in Wayne County Medical Examiner's cases. J. Forensic Sci. 1990;35:591–600.

107. Mclaughlin CR, Daniel J, Joost TF. The relationship between substance use, drug selling,

and lethal violence in 25 juvenile murderers. J. Forensic Sci. 2000;45:349–53.

108. Rogers C. Gang-related homicides in Los Angeles County. J. Forensic Sci. 1993;38:831–4.

109. Sharps PW, Campbell J, Campbell D, Gary F, Webster D. The role of alcohol use in intimate partner femicide. Am. J. Addict. 2001;10:122–35.

110. Varano SP, McCluskey JD, Patchin JW, Bynum TS. Exploring the drugs-homicide connection. J. Contemp. Crim. Justice. 2004;20:369–92.

111. Varano SP, Cancino JM. An empirical analysis of deviant homicides in Chicago. Homicide Stud. 2001;5:5–29.

112. Welte JW, Abel EL. Homicide: drinking by the victim. J. Stud. Alcohol. 1989;50:197–201.

113. Albrektsen SB, Thomsen JL, Aalund O, Breiting VB, Danielsen L, Helweg-Larsen K, et al. Injuries due to deliberate violence in areas of Denmark. IV. Alcohol intoxication in victims of violence. The Copenhagen Study Group. Forensic Sci. Int. 1989;41:181–91.

114. Allgulander C, Nilsson B. Victims of criminal homicide in Sweden: a matched case-control study of health and social risk factors among all 1,739 cases during 1978-1994. Am. J. Psychiatry. 2000;157:244–7.

115. Lindqvist P. Homicides committed by abusers of alcohol and illicit drugs. Br. J. Addict. 1991;86:321–6.

116. Davies M, Mouzos J. Homicide in Australia: 2005-2006 National Homicide Monitoring Program annual report. Canberra; 2007.

117. Dooley E. Homicide in Ireland 1972-1991. Dublin; 1995.

118. Dooley E. Homicide in Ireland 1992-1996. Dublin; 2001.

119. Lerer LB. Women, homicide and alcohol in Cape Town, South Africa. Forensic Sci. Int. 1992;55:93–9.

120. Duflou JA, Lamont DL, Knobel GJ. Homicide in Cape Town, South Africa. Am. J. Forensic Med. Pathol. 1988;9:290–4.

121. Lunetta P, Penttilä A, Sarna S. The role of alcohol in accident and violent deaths in Finland. Alcohol. Clin. Exp. Res. 2001;25:1654–61.

122. Virkkunen M. Alcohol as a factor precipitating aggression and conflict behaviour leading to homicide. Br. J. Addict. Alcohol Other Drugs. 1974;69:149–54.

123. Hocking MA. Assaults in south east London. J. R. Soc. Med. 1989;82:281–4.

124. Hulse GK, English DR, Milne E, Holman CD. The quantification of mortality resulting from the regular use of illicit opiates. Addiction. 1999;94:221–9.

125. Bradshaw D, Groenewald P, Laubscher R, Nannan N, Nojilana B, Norman R, et al. Initial burden of disease estimates for South Africa, 2000. South African Med. J. 2003;93:682–8.

126. Reza A, Mercy JA, Krug E. Epidemiology of violent deaths in the world. Inj. Prev. 2001;7:104–11.

127. Leroux LC, Smith LS. Violent deaths and alcoholic intoxication. J. Forensic Med.

1964;11:131–47.

128. Moar JJ. Homicidal penetrating incised wounds of the thorax. An autopsy study of 52 cases. South African Med. J. 1984;65:385–9.

129. Mathews S, Abrahams N, Jewkes R, Martin LJ, Lombard C. Alcohol use and its role in female homicides in the Western Cape, South Africa. J. Stud. Alcohol Drugs. 2009;70:321–7.

130. Dhossche DM, Meloukheia AM, Chakravorty S. The association of suicide attempts and comorbid depression and substance abuse in psychiatric consultation patients. Gen. Hosp. Psychiatry. 2000;22:281–8.

131. Murphy GE. Suicide and Substance Abuse. Arch. Gen. Psychiatry. 1988;45:593.

132. Rich C, Fowler R, Young D. Substance Abuse and Suicide. The San Diego Study. Ann. Clin. Psychiatry. 1989;1:79–85.

133. Mann RE, Zalcman RF, Smart RG, Rush BR, Suurvali H. Alcohol Consumption, Alcoholics Anonymous Membership, and Suicide Mortality Rates, Ontario, 1968-1991. J. Stud. Alcohol. 2006;67:445–53.

134. Maloney E, Degenhardt L, Darke S, Mattick RP, Nelson E. Suicidal behaviour and associated risk factors among opioid-dependent individuals: a case-control study. Addiction. 2007;102:1933–41.

135. Gunnell D, Ashby D. Antidepressants and suicide: what is the balance of benefit and harm. BMJ. 2004;329:34–8.

136. Reeves RR, Ladner ME. Antidepressant-induced suicidality: an update. CNS Neurosci. Ther. 2010;16:227–34.

137. Dhossche DM, Rich CL, Isacsson G. Psychoactive substances in suicides. Comparison of toxicologic findings in two samples. Am. J. Forensic Med. Pathol. 2001;22:239–43.

138. Haum A, Perbix W, Häck HJ, Stark GB, Spilker G, Doehn M. Alcohol and drug abuse in burn injuries. Burns. 1995;21:194–9.

139. Swenson JR, Dimsdale JE, Rockwell E, Carroll W, Hansbrough J. Drug and alcohol abuse in patients with acute burn injuries. Psychosomatics. 1991;32:287–93.

140. Howland J, Hingson R. Alcohol as a risk factor for injuries or death due to fires and burns: review of the literature. Public Health Rep. 1987;102:475–83.

141. Barillo DJ, Goode R. Substance abuse in victims of fire. J. Burn Care Rehabil. 1996;17:71–6.

142. Bruck D, Ball M, Thomas IR. Fire fatality and alcohol intake: analysis of key risk factors. J. Stud. Alcohol Drugs. 2011;72:731–6.

143. Lunetta P, Smith GS, Penttilä A, Sajantila A. Unintentional drowning in Finland 1970-2000: a population-based study. Int. J. Epidemiol. 2004;33:1053–63.

144. Ahlm K, Saveman B-I, Björnstig U. Drowning deaths in Sweden with emphasis on the presence of alcohol and drugs – a retrospective study, 1992–2009. BMC Public Health. 2013;13:216.

145. Athanasiadis L. Drugs, alcohol and violence. Curr. Opin. Psychiatry. 1999;12:281–6.

146. Haggård-Grann U, Hallqvist J, Långström N, Möller J. The role of alcohol and drugs in triggering criminal violence: a case-crossover study*. Addiction. England; 2006;101:100–8.

147. Weiner MD, Sussman S, Sun P, Dent C. Explaining the link between violence perpetration, victimization and drug use. Addict. Behav. 2005;30:1261–6.

148. Ousey GC, Lee MR. Examining the conditional nature of the illicit drug market-homicide relationship: a partial test of the theory of contingent causation. Criminology. 2002;40:73–102.

149. Tardiff K, Marzuk PM, Lowell K, Portera L, Leon AC. A study of drug abuse and other causes of homicide in New York. J. Crim. Justice. 2002;30:317–25.

150. Ellickson PL, Tucker JS, Klein DJ. Ten-year prospective study of public health problems associated with early drinking. Pediatrics. 2003;111:949–55.

151. Scott KD, Schafer J, Greenfield TK. The role of alcohol in physical assault perpetration and victimization. J. Stud. Alcohol. 1999;60:528–36.

152. Wells S, Graham K. Aggression involving alcohol: relationship to drinking patterns and social context. Addiction. 2003;98:33–42.

153. Ramstedt M. Population drinking and homicide in Australia: A time series analysis of the period 1950-2003. Drug Alcohol Rev. 2011;30:466–72.

154. Stickley A, Razvodovsky Y. The effects of beverage type on homicide rates in Russia, 1970-2005. Drug Alcohol Rev. 2012;31:257–62.

155. Pridemore WA. Vodka and Violence: Alcohol Consumption and Homicide Rates in Russia. Am. J. Public Health. 2002;92:1921–30.

156. Matzopoulos R. Violent Deaths in SA: The 2003 National Injury Mortality Surveillance System. SA Crime Q. 2005;29–36.

157. Dresen S, Ferreirós N, Gnann H, Zimmermann R, Weinmann W. Detection and identification of 700 drugs by multi-target screening with a 3200 Q TRAP LC-MS/MS system and library searching. Anal. Bioanal. Chem. 2010;396:2425–34.

158. StataCorp. Stata Statistical Software: Release 14. College Station: TX: StataCorp LP; 2015.

159. Sheehan CM, Rogers RG, Williams GW, Boardman JD. Gender differences in the presence of drugs in violent deaths. Addiction. 2013;108:547–55.

160. da Silva AA, Bester MJ, Pretorius E. Effects of Mandrax and Cannabis on the cellular function of chick embryonic neurons. Environ. Toxicol. Pharmacol. 2007;23:82–8.

161. Williams ME, Davis SS, Poxon R, Kendall MJ, Mitchard M. The influence of diphenhydramine on the absorption of methaqualone in man. Br. J. Clin. Pharmacol. 1974;1:259–64.

162. Peltzer K, Ramlagan S, Johnson BD, Phaswana-Mafuya N. Illicit drug use and treatment in South Africa: A review. Subst. use misuse. 2010;45:2221–43.

163. Darke S, Kaye S, McKetin R, Duflou J. Major physical and psychological harms of methamphetamine use. Drug Alcohol Rev. 2008;27:253–62.

164. Moore TM, Stuart GL. A review of the literature on marijuana and interpersonal violence. Aggress. Violent Behav. 2005;10:171–92.

165. Parry CDH, Plüddemann A, Donson H, Sukhai A, Marais S, Lombard C. Cannabis and other drug use among trauma patients in three South African cities, 1999-2001. South African Med. J. 2005;95:429–32.

166. Tennant FS. Complications of methaqualone-diphenhydramine (Mandrax R) abuse. Br. J. Addict. Alcohol Other Drugs. 1973;68:327–30.

167. Hindmarsh KW, Wallace SM, Schneider CB, Korchinski ED. Methaqualone-diphenhydramine interaction study in humans. J. Pharm. Sci. 1983;72:176–80.

168. Hindmarsh KW, Hamon NW, LeGatt DF, Wallace SM. Effect of diphenhydramine on methaqualone metabolism: an in vitro study. J. Pharm. Sci. 1978;67:1547–50.

169. Edwards GI. Drugs on your streets (illustrated). The Rosen Publishing Group; 1997.

170. Rataemane S, Rataemane L. Alcohol in South Africa. Int. J. Drug Policy. 2006;17:373–5.

5. Budget

Resource	Item	Cost (Rands)
Administrative	Printing & Copying	200.00
Travel	Fuel	400.00
Consumables	Plastic containers (x2)	466.85
	Evidence bags	Provided
	15mL Falcon tubes	1072.00
	50mL Falcon tubes	1262.00
	4mL Grey-top tubes	474.00
	10mL Red-top tubes	162.12
	Envelopes	Provided
	Markers	Provided
	Self-stick Labels	Provided
	Cello tape	Provided
	Scissors	Provided
	Ice packs	Provided
	1.5mL Eppendorf tubes	275.00
	Pipettes	321.21
	Acetonitrile	1718.00
	De-ionized water	Provided
	TOTAL	R 5950.91

6. Ethics Approval Letters

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

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
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08 July 2015

HREC REF: 324/2015

Ms B Davies
Forensic Medicine & Toxicology
Falmouth Building
Entrance 3, level 5

Dear Ms Davies

PROJECT TITLE: A POST-MORTEM TOXICOLOGICAL INVESTIGATION: UNDERSTANDING THE ROLE OF DRUGS OF ABUSE IN VIOLENT FATALITIES IN CAPE TOWN, SOUTH AFRICA (MPhil-candidate K Auckloo)

Thank you for your considered response to the Faculty of Health Sciences Human Research Ethics Committee received on 7 July 2015.

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

Approval is granted for one year until the 30th July 2016.

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

Please quote the HREC REF in all your correspondence.

We acknowledge that the student, Kathrina Auckloo will also be involved in this study.

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

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
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 Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH

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2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki 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.

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences

Human Research Ethics Committee

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11 September 2015

HREC REF: 324/2015

Ms B Davies
Forensic Medicine & Toxicology
Level 1, Entrance 3

Dear Ms Davies

PROJECT TITLE: A POST-MORTEM TOXICOLOGICAL INVESTIGATION: UNDERSTANDING THE ROLE OF DRUGS OF ABUSE IN VIOLENT FATALITIES IN CAPE TOWN, SOUTH AFRICA (MPhil-candidate K Auckloo)

Thank you for your letter to the HREC, requesting that delayed consent from the next-of-kin be allowed in this study.

The HREC are satisfied that that consent process may be delayed, for instances where the autopsy is conducted prior to the identification process, and it is therefore not possible to obtain informed consent from the next-of-kin prior to taking samples.

The HREC note that samples will be stored until informed consent has been obtained from the next-of-kin. If delayed consent is unsuccessful, or the case remains unidentified, the samples will not be used for research purposes, and will be destroyed.
However, for this amendment to be formally approved, please complete an FHS006 form.

Please also provide a separate informed consent document for the delayed consent process. This informed consent document must clearly indicate that samples have already been taken and stored, and why the samples have been taken prior to consent being obtained, i.e. not just that the autopsy has happened already but that the samples degrade or that samples were taken to avoid additional cutting of the decedent, etc.

Please retain the original version of the informed consent document that was approved by the HREC for next-of-kin of the decedent who give consent prior to the collection of samples.

Please quote the HREC REF in all your correspondence.

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

Yours sincerely

Signed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE