Toxicological Findings in Fatal Road Traffic Accidents in Cape Town: A Pilot Study

By

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RESEARCH SUMMARY

Introduction: Road traffic accidents (RTAs) and associated morbidity and mortality are a global public health burden. Literature reports on an association between drugs and/or alcohol intoxication and traffic collisions. In South Africa (SA), where drug use and abuse are prevalent, annual RTAs rates are higher than the average global burden. Toxicological analyses in cases of RTA fatalities are not performed routinely in SA (apart from alcohol analysis), thus understanding the burden of other drug impairment on road traffic deaths is limited.

Aim: A prospective toxicological analysis was performed in a cohort of road traffic fatality cases (drivers, passengers, pedestrians, motorcyclists and cyclists) from Salt River Mortuary in Cape Town, SA. The objectives were to perform drug screening in these cases to preliminary investigate detected substances as well as to evaluate the demographics and circumstances of death of the aforementioned cases.

Methods: A systematic review was first performed to investigate the prevalence of drugs in internationally reported RTA fatalities.
For the prospective study, post-mortem specimens including blood, vitreous humor, urine and bile were collected from cases in which next-of-kin consent was obtained. All samples were analysed using liquid chromatography-quadrupole time-of -flight mass spectrometry (LC/QTOF-MS).

Results: Thirty cases were analysed over 3 months, of which most were male, pedestrians and between the age group of 31-40 years. The most prevalent cause of death was multiple blunt force injuries to the body. Single vehicle crashes were predominant particularly among the pedestrians and motorcyclists whereas drivers were mostly involved in multiple vehicle crashes. Substances (other than ethanol) were detected in 90% (n=27) of the cases. A broad range of drug groups were detected, and the most prevalent specific legal substances were caffeine (66.7%) and nicotinamide (53.3%) and illegal substances were methaqualone (10.0%) and methamphetamine (6.67%). Multiple cases indicated the detection of impairing substances even if consumed therapeutically, such as codeine, chlorpheniramine, diphenhydramine and zopiclone.

Discussion: This study was the first to the author's knowledge to report on prospective toxicological findings in road traffic accident cases in Cape Town. Although this was a pilot study, the results were in line with findings from other international studies, together with findings of prominent abused drugs within Western Cape (e.g. methaqualone and methamphetamine). While this study made no inferences of drug intoxication to cause of death, it has set a basis for future

research in this topic and the development of a standardised protocol for the routine analyses of such cases in SA.

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LIST OF ABBREVIATIONS

ADHD	Attention Deficit Hyperactivity Disorder
AOD	Alcohol or drug
AXIS	Appraisal Tool for Cross-Sectional Studies
BAC	Blood alcohol concentration
CINAHL	Cumulative Index of Nursing and Allied Health Literature
CNS	Central nervous system
COD	Cause of death
DUI	Driving under the influence
ESI	Electron Spray Ionisation
FCL	Forensic Chemistry Laboratory
FPO	Forensic Pathology Officer
FPS	Forensic Pathology Services
GA	Gauteng
GC	Gas Chromatography
GC-FID	Gas Chromatography - Flame Ionisation Detection
GC-MS	Gas Chromatography Mass Spectrometry
GC-NPD	Gas Chromatography - Nitrogen Phosphorous Detector
GDP	Gross domestic product
GHB	Gammahydroxybuturate
HIC	High income country
HPLC- DAD	High-Performance Liquid Chromatography with Diode-Array Detection
HPLC	High Performance Liquid Chromatography
HREC	Human Research Ethics Committee
JBI	Joanna Briggs Institute
Km/h	Kilometers per hour
KZN	Kwazulu Natal
LC	Liquid Chromatography
LC-MS	Liquid Chromatography Mass Spectrometry
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LC-QTOF MS	Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry
LMIC	Low and middle-income countries

MDMA	Methylenedioxymethamphetamine
MeSH	Medical Subject Headings
MPA/B	Mobile Phase A/B
MVC	Multiple vehicle collisions
NW	North West
OTC	Over the counter drugs
PICO	Population, Intervention, Comparison, Outcome
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RTA	Road traffic accidents
RTF	Road traffic fatalities
RTI	Road traffic injury
RTMC	Road Traffic Management Centre
SA	South Africa
SACENDU	South African Community Epidemiological Network on Drug Use
SAPS	South African Police Services
SRM	Salt River Mortuary
SVC	Single vehicle collisions
THC	Tetrahydrocannabinol
THCCOOH	11-nor-9-carboxy-delta 9-tetrahydrocannabinol
THCOH	11-hydroxy tetrahydrocannabinol
TLC	Thin Layer Chromatography
UCT	University of Cape Town
UPLC	Ultra Performance Liquid Chromatography
UVS	Ultraviolet spectrophotometry
VH	Vitreous humor
WC	Western Cape
WHO	World Health Organization

Chapter 1: Background

Road traffic accidents (RTAs) are a global concern with an associated high public health burden. Road users are at the risk of injury and fatality based on multiple risk factors. This chapter introduces the road user and explains the risk factors which may predispose them to RTAs. In addition, it provides contextual information on the role of alcohol and drugs and their involvement in the injury and/or death of road users.

1.1 Road Traffic Accidents: A Global Public Health Problem

This section defines and discusses the road user, road traffic injuries and fatalities in which they may be involved and the extent in which RTAs are a global burden to public health systems.

1.1.1 Road Users

All road users worldwide are at risk of RTAs resulting in injury and/or death, affected by varying environmental, safety and individual risk factors. A road traffic crash or accident is a collision or incident, which may or may not lead to injury and/or death, in which at least one moving (motorised or non-motorised) vehicle makes contact with other road users or stationary objects in a public or private road [1]. A road user refers to an individual using motorised or non-motorised transport on any part of the road system [2]. Motorised transportation includes two-or-more wheeled motorised vehicles powered by a motor engine, whereas non-motorised transportation refers to a transport method that does not need a motor to generate energy (e.g. cycling) [3] (Table 1.1).

Table 1.1: Types of road users based on the method of transportation.

Motorised Transport	User	Non-Motorised Transport	User
· **Two-wheeled vehicles** (e.g. motorcycle, moped)	*Drivers Passengers*	· **Walking**	*Pedestrians*
· **Three-wheeled vehicles** (e.g. scooter taxi)	*Drivers Passengers*	· **Two-wheelers** (e.g. bicycle)	*Cyclists*
· **Four-wheeled vehicles** (e.g. car)	*Drivers Passengers*	· **Three-wheelers** (e.g. Tricycle)	*Cyclists*
· **Others** (e.g. truck, lorry, bus)	*Drivers Passengers*	· **Human- and animal-powered vehicles (HAPV)** (e.g. animal-drawn carts)	*Drivers Passengers*

Road users are classified into five groups, namely drivers, passengers, pedestrians, motorcyclists and cyclists [4]. The last three groups are considered to be especially vulnerable road users based on the degree of external protection (e.g. vehicle without a shell), task capability (e.g. limitations due to disability, social or cultural circumstances), and resilience (e.g. novice or elderly drivers). Vulnerable road users usually bear the greatest burden of injury in RTAs [5].

1.1.2 Road Traffic Injuries

Over 5 million individuals die every year as a result of injuries, which accounts for approximately 10% of all deaths globally [6]. In 2012, approximately a quarter of these injury related deaths were attributed to road traffic injuries (RTIs); one of the three leading injury-related causes of death (Figure 1.1) [7]. Recognised as a major global public health concern [8], RTIs are defined as fatal or non-fatal injuries as a result of a traffic accident [1]. The World Health Organization (WHO) estimates about 20 to 50 million individuals are injured (or disabled) every year in road traffic crashes; the wide range due to variability of reporting or lack thereof [9].

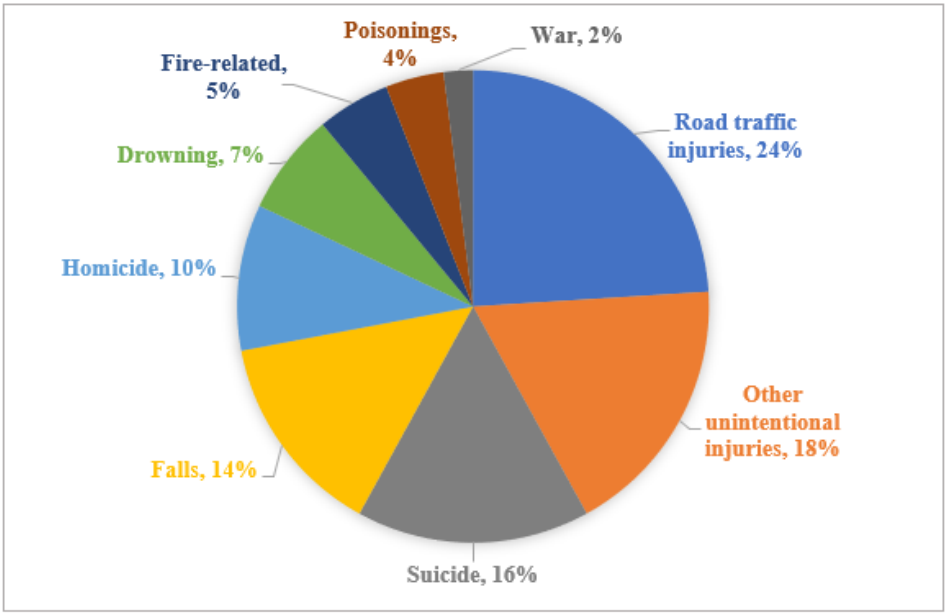


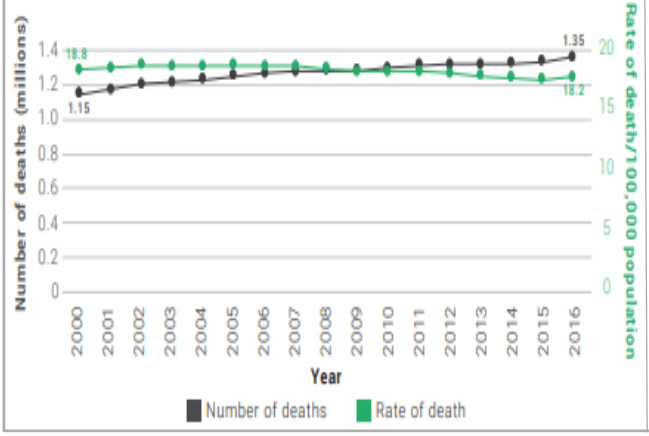
Figure 1.1: Distribution of the causes of injury mortality worldwide, 2012 [6].

Strategies to prevent road traffic crashes and resulting injuries require concerted and multi-disciplinary efforts for effective and sustainable solutions. Unsurprisingly, road traffic injuries greatly affect vulnerable road users, especially children and young adults [7]. This burden has become the principal global cause of injury (and death) among the youth aged 5 to 29 years old worldwide [10] , warranting the need for greater attention in the current child health agenda.

1.1.3 Road Traffic Fatalities

RTIs have other consequences which affect both the individuals involved and their families. Non-fatal health consequences include injuries leading to chronic treatment and hospitalization, mental health issues and changes in behavioural practices. The most devastating consequence though, is, one which leads to death [11]. Road traffic fatality (RTF) is usually reported as such when an individual dies within 30 days of the traffic crash and the cause of death (COD) was directly related to the collision [1]. Fatal traffic crashes have increased to approximately 1.35 million a year accounting for 2.5% of all deaths and becoming the 8th leading COD for people of all ages, globally (Figure 1.2) [10]. Without new initiatives and increased efforts to reduce these accidents, the number of traffic deaths is expected to rise significantly right through to 2020 [12]. This is particularly the case in low and middle income countries (LMICs) where there is a higher variation and level of traffic mix (i.e. different modes of transport, motorised and non-motorised, that share the same road network) and poor separation of the vulnerable, non-motorised groups from the fast moving, motorised vehicles [13].

A



B

Rank	Cause	% of total deaths
1	Ischemic heart disease	16.6
2	Stroke	10.2
3	Chronic obstructive pulmonary disease	5.4
4	Lower respiratory infections	5.2
5	Alzheimer's diseases and other dementias	3.5
6	Trachea, bronchus, lung cancers	3.0
7	Diabetes mellitus	2.8
8	Road traffic injuries	2.5
9	Diarrheal disease	2.4
10	Tuberculosis	2.3

Figure 1.2: Global burden of road traffic deaths. (A) The number of road traffic deaths in millions from 2000 to 2016. (B) The leading causes of death for all ages in 2016 [9].

1.2 Risks of Road Traffic Crashes

This section speaks to the risks that road users are exposed to and hones in on the human risk factor of driving impairment due to alcohol and drugs of use and abuse.

1.2.1 Understanding the risks

The risks in which road users are exposed to in road traffic are comprised into four major aspects: i) exposure- the need to travel and movement in different parts of a transport system by different road user groups, ii) crash involvement given a particular exposure, iii) crash severity and iv) post-crash outcome (Table 1.2) [13].

Table 1.2: Risk factors for road traffic injury and mortality [11].

i) Exposure:	**ii) Crash involvement:**
· Demographics · Socioeconomic factors · Use of land and planning of road layout which effects distances of trip and mode of transport · Variation and traffic mix of motorised vehicles with non-motorised users	· Travelling at high or inappropriate speeds · **Use of alcohol and recreational or medicinal drugs** · Vehicle defects such as brakes or tires · Environmental factors affecting visibility of the road and other vehicles · Road defects such as road maintenance
iii) Crash severity:	**iv) Post-crash injury:**
· Lack of seat belt and child restraint use · Helmets and protective gear not worn by two wheeled road users · Human tolerance factors · **Use of alcohol and recreational or medicinal drugs** · Crash protection for vehicle occupants and those outside vehicle	· Fire caused by the collision · Delay in detecting crash · Presence/leakage of hazardous materials · Challenges in rescuing, extracting or evacuating vehicle (car, bus, coach) occupants · Lack of necessary pre-hospital care or delay in getting to a hospital · Lack of appropriate hospital care and treatment

The risk of RTIs and/or death is clearly multifactorial. A major contributor to road traffic crash involvement is factors affecting the individual, of particular note is impairment by alcohol and/or recreational or medicinal drugs (Table 1.2) [14]. The use of impairing substances by drivers and pedestrians may affect cognitive function and judgement. This in turn influences both crash risk and crash consequence, while increasing crash risks for others on the road [15].

1.2.2 Implications of Alcohol

Driving under the influence of alcohol has been reported to greatly increase the risk of being involved in traffic accidents [16] and it has been shown that drivers who have consumed alcohol are at an increased risk than those with who have not [17]. Due to the potential of impairment,

alcohol was found to increase the risk of crash involvement, crash severity, and/or post-crash injury outcome by affecting the drivers' choice of speed [13].

It is estimated that alcohol-related road fatalities comprise approximately between 5-35% (with a weighted average of 21.8%) of all RTFs globally [18, 19]. Any amount of alcohol in a driver's system has been shown to impair their driving behaviour, and there is a rapid and exponential increase in risk for blood alcohol concentration (BAC) levels exceeding legal driving limits for the general driving population worldwide [20]. The risk of an alcohol influenced crash varies with drinking experience and age, and the crash rates of younger aged males (16-20 years) was at least 3 times that of men aged 25 years and above at every BAC level [16].

In many low-income countries, there is a lack of equipment and human resources to routinely monitor the alcohol levels in drivers even though legal limits exist [21] and this makes it somewhat of a challenge to perform studies in which data can be compared across countries to understand the full status of the situation on a global level. In addition, many alcohol-related road fatalities involving pedestrians and cyclists, may not be documented if not considered as 'active participants' due to the absence of a 'legal limit' for these road users. Moreover, data on 'drink-driving' – driving a motor vehicle under the influence of alcohol – remains scarce in multiple countries. Such information is crucial in understanding the extent of this public health issue as well as to evaluate the impact of prevention efforts.

1.2.3 Implications of Drugs of Abuse (other than Alcohol)

Similar to alcohol, any other substance that affects the central nervous system (CNS) (e.g. medicinal and/or recreational drugs) as well as cognition, behaviour and motor control, can contribute to road traffic crashes [22]. Their effects, however, on driving impairment and road crash involvement are not yet well understood than those of alcohol [23]. Establishing the role of causation versus contribution of drug use to a crash is therefore challenging, especially if other risk factors are involved [24].

The primary reasons for such complexities include the following: (a) unlike alcohol, a lot of drugs do not exhibit a direct relationship between drug concentration in blood and level of impairment [25], (b) drivers with medical conditions may be safer driving having taken their medication that may otherwise impair others (for example, schizophrenic patients prescribed antipsychotic drugs) [26], (c) the differences in which individuals respond to particular drugs at different doses and administration routes [13], (d) the differing short and long term effects of certain drugs [27], and

(e) antagonistic or synergistic effects of different drugs used together (e.g. concomitant use of drugs and alcohol) [28].

There is still limited global information on the extent and role of substance use in cases of 'drugged-driving'. These also rely on improving our understanding of the effects of drug use on driving capacity, especially poly-drug use. Poly-drug use increases the risk of traffic accidents greatly [29] and this issue of drug involvement in traffic accidents needs urgent attention. In addition to this, there is increased complexity in interpreting drug concentrations in biological samples obtained from deceased individuals, where post-mortem factors may alter their concentrations. Improving our understanding of the presence of drugs in cases of road traffic deaths as well as improving our interpretive capacities of understanding the role of these drugs in the death is essential moving forward.

1.3 Conclusion

A systematic review was conducted to provide greater insight into the information available globally on the role of toxicology in road traffic deaths and the reported prevalence of substances in RTFs. This spoke to the availability of this data in developing countries, such as South Africa (SA), and developed a framework for the prospective investigation into drugs detected in a deceased cohort at a metropolitan mortuary in Cape Town.

Chapter 2: Systematic Literature Review

A Forensic Toxicology Perspective on the Prevalence of Drugs of Abuse in Fatal Road Traffic Accidents using a Systematic Literature Review

2.1 Abstract

Background: Involvement of drugs in road traffic accidents (RTAs) is an important, yet not well understood problem, which is associated with a high global morbidity and mortality. There is also limited information on this issue in the context of the forensic services case load to which RTAs contribute significantly. The aim of this review was thus to systematically investigate the global prevalence of drugs of use and abuse reported in fatal RTAs.

Methods: The review was conducted according to PRISMA guidelines in which original articles were searched for in PubMed, Scopus, Web of Science and CINAHL. Grey literature sources included Google Scholar, WorldCat and ProQuest Dissertation databases. The reference lists were all hand searched and the data was extracted by one reviewer with quality checks by two other reviewers. The data was qualitatively summarised based on the outcomes.

Results: Of the 431 eligible articles, 42 were included in the review of which most were of poor to fair quality based on the quality assessment. Studies originated primarily from Europe and the most common road user group investigated were drivers of motorised vehicles. Overall, the prevalent substances detected were alcohol, followed by cannabis, benzodiazepines, stimulants and opioids.

Conclusions: Studies were largely reported from developed countries. Insightful information was provided on the drug prevalence in fatal RTAs and the need to intensify enforcement action on drugged driving. More research is needed to investigate drugs in the vulnerable road user groups as well as to increase efforts to alleviate the limited resources and research capacity in developing countries where fatal RTAs are a significant burden.

2.2 Introduction

Fatal RTAs and collisions continue to be a common occurrence and are one of the leading cause of mortality worldwide [14]. Forensic science plays a prominent role in the case investigation of human deaths, especially in determining the cause and manner of death of road traffic collision victims [30]. As part of this investigation, forensic toxicology - which includes the ancillary analysis for alcohol and drugs (therapeutic or recreationally used) in biological specimens - is crucial in understanding the possible contribution of substance impairment to the road traffic death [30].

Medico-legal road traffic cases can contribute substantially to a forensic toxicology laboratory's workload, allowing for numerous retrospective studies and available data. Many have reported on the potential role of alcohol, drugs of use and abuse (e.g. cannabis) [31, 32] or specific drug classes (e.g. benzodiazepines and amphetamines) in RTAs [33, 34]. The involvement of alcohol on the possible impairment of the driver has been most highly studied and reported on [15], however the effects of illicit and prescribed drugs on road users has received much less attention [35] .

Currently, there is a lack of systematic evidence on the prevalence of drugs (other than alcohol) in fatal RTAs worldwide. This review intends to add to the literature and fill in the knowledge gap by providing an understanding of the role, effects and extent of drug involvement in such fatalities in the global context. Furthermore, the review will highlight the practice of routine toxicological testing in RTFs and its role in potential surveillance, intervention and prevention strategies.

2.3 Objectives

This systematic review was directed by the following research question: *"What is the prevalence of drugs of abuse in global road traffic fatalities?"*. With the aim to investigate the global prevalence of substances (alcohol and other drugs) commonly detected in fatal RTAs, this literature review provides a descriptive and comparative study of toxicological findings in victims of RTFs with the objective to assess the use and extent of forensic toxicological analyses in road traffic case investigations.

2.4 Methods

This review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines [36].

2.4.1 Criteria for Considering Studies

2.4.1.1 Types of Studies

The inclusion and exclusion criteria for eligible studies in this review were:

Table 2.1: Inclusion and exclusion criteria for review articles.

Inclusion	Exclusion
i. Conducted anywhere in the world i.e. no restriction with regards to the geographical distribution to obtain a global overview. ii. Studies which reported on the prevalence, incidence and/or presence of any drugs in ante- or post mortem samples submitted to forensic toxicology laboratories to assist in the determination of the contribution of substances and impairment to the fatal RTAs iii. Studies which presented quantitative prevalence information in the form of percentage or proportion. iv. No timeframe restriction was applied to include all potentially relevant research study on the topic. v. Cross-sectional study design, which was most appropriate to address the review question. vi. Research studies reported in any language were included to avoid language bias. vii. Studies where road users were of any population group and of all ages.	i. Non-original primary research such as opinions, commentaries, perspectives, and correspondences. ii. Single case reports which would not be representative of road user populations. iii. Research studies specific to certain drug class(es) or drug compound(s) which would bias the evidence towards that specific drugs being investigated.

2.4.1.2 Population Types

The review included road-traffic deaths of all types of road users, namely; drivers of motor vehicles, passengers, pedestrians, bicyclists, and motorcyclists.

2.4.1.3 Types of Outcome Measures

The outcomes chosen were deemed most forensically valuable in assessing the involvement of drugs of abuse in RTFs. The primary outcomes were: (a) the death of the road users, and (b) the practice of conducting a forensic toxicology investigation in such deaths. Secondary outcomes include the detection and identification of drugs of use and abuse in RTFs. These included (c) the toxicological general screening of substances, (d) the quantification of detected substances, and (e) the reported prevalence count.

2.4.2 Search Methods for Identification of Studies

2.4.2.1 Information Sources

For the identification of relevant prevalence studies in the field, four databases (PubMed/Medline, Scopus, Web of Science and CINAHL) were comprehensively searched for publications from inception to the 16th August 2018. Similarly, WorldCat, Google Scholar and ProQuest Dissertations were searched as grey literature sources to minimise publication bias. The reference lists of retrieved studies were hand-searched to ensure no relevant articles were overlooked. Overall, the search screened from the earliest published and un-published articles, working papers, dissertations, reports and other grey literature documented.

2.4.2.2 Search Strategy

Based on the PICO (Population, Intervention, Comparison, Outcome) framework for evidence-based research [37], the following keywords were defined: *'road users'*, *'substances or drugs of abuse'*, and *'fatal road traffic accidents or road traffic fatalities*. These pre-defined keywords were used to develop a broad range of medical subject headings (MeSH) terms in order to ensure a comprehensive and extensive search. For each database, combinations of the keywords and MeSH terms were used for all searches (Appendix A and B). Titles and abstracts of all records identified were screened for all pre-defined keywords. No language or date restrictions were applied in all searches.

2.4.3 Data Collection & Analysis

2.4.3.1 Study Collection

The publications and articles identified as a result of the search strategy were independently screened for eligibility by one review author (NS) and any uncertainty on eligibility was resolved through discussion with two other reviewers (KA and BD). Subsequently, the full text of the included screened studies were assessed using the inclusion and exclusion criteria by one review author (NS) and consensus on any uncertainties was reached following discussion with the other two reviewers (KA and BD).

2.4.3.2 Data Extraction & Management

The JBI Data Extraction Form for Prevalence and Incidence Studies (Appendix C), a standardised data extraction form [38] , was used as a template and adapted accordingly to accommodate for the outcomes associated with the review question. One reviewer (NS) independently collected the

following information from each included article: (i) name of author(s), (ii) title, (iii) year of publication, (iv) country, (v) study time period, (vi) aims & objectives, (vii) research question/hypothesis, (viii) population of interest, (ix) sample size, (x) methods of toxicological analysis, (xi) statistical significance, (xii) measurement instruments, (xiii) outcome measures (prevalence), (xiv) demographics, (xv) limitations, and (xvii) ethical considerations. Consensus was achieved on any disagreements regarding extracted data among all three reviewers (NS, KA, BD). When a study did not describe any of the above information of interest the original author of the study was contacted to request the necessary information.

2.4.3.3 Quality Assessment of Individual Studies

A descriptive quality assessment of the included articles was conducted using the AXIS (Appraisal Tool for Cross-Sectional Studies) tool, a critical appraisal tool developed to assess the quality of cross-sectional studies across disciplines (Appendix D) [39]. The AXIS tool addressed the study design and reporting quality as well as the risk of bias in cross-sectional studies, hence aiding the inclusion of such study designs in the systematic review. The tool's focus is largely on the presented methods and results and provide the opportunity for reviewers to assess each individual aspect of study design to give an overall assessment quality rating. The tool does not use a numerical scale to assess the overall quality of a study but rather, allows for more flexibility in considering the quality of reporting and the risk of bias when reviewers make judgements on the quality of the studies [39]. The perceived limitation on the degree of subjectivity in assessing the quality of the studies was mitigated by having three reviewers (NS, KA and BD) independently assess the quality of the included studies. Any disagreements were resolved by discussion to reach a consensus.

2.5 Findings

2.5.1 Search Result

From the electronic databases and grey literature sources searched 639 publications were identified in total, of which 168 were duplicates. Therefore, 471 publications were screened for initial eligibility based on the title and abstract. Subsequently, 431 citations were excluded, narrowing the number of articles eligible for full-text review to 40 (Figure 2.1). Upon assessing the reference lists of reviewed articles, an additional 11 articles were identified. Out of the total 51 potential studies, 9 were excluded and 42 studies (Appendix E) were included in this review's quantitative analysis.

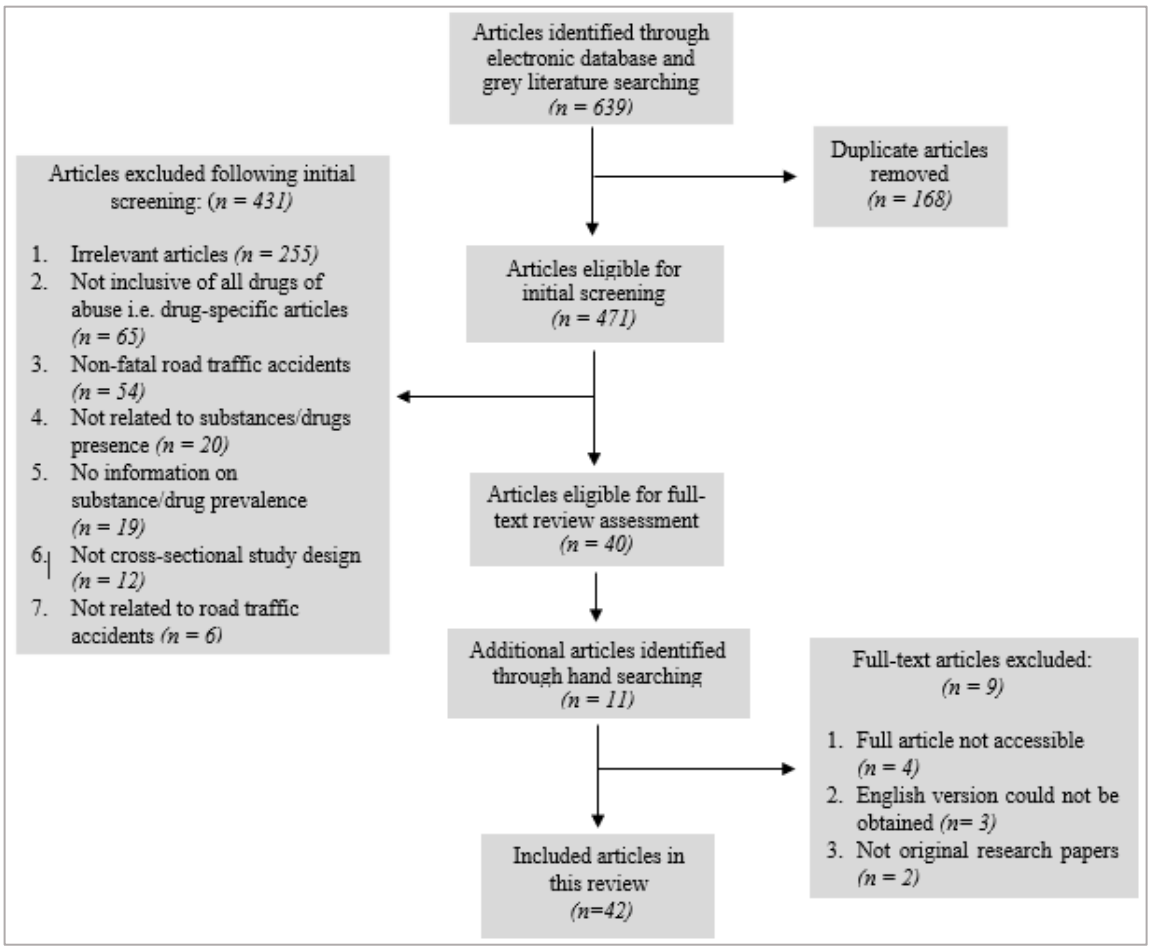


Figure 2.1: Flow diagram illustrating the literature search strategy.

2.5.2 Quality Assessment

As shown in Table 2.2, most of the included studies were of fair or poor quality, with less than a third deemed to be of good quality.

Table 2.2: Quality assessment of included articles.

Quality Rating	Main Features	Number of articles (%)	Citations
Good	Methods were sound and applicable and clear results were provided in relation to the aim of the study.	11 (26.2)	[40-50]
Fair	Insufficient description of the methods provided but adequate description of the results which were internally consistent.	16 (38.1)	[51-66]
Poor	Older studies which lacked sufficient descriptions of the methods as well as descriptions of basic descriptive data in the results.	15 (35.7)	[67-81]

2.5.3 Global Distribution

The included studies originated from 6 continents, with most coming from Europe (45.2%), and North America (28.6%) (Table 2.3). Most of the studies from the same country (e.g. Norway, Sweden and the United States) collected data from the same database but during different study periods, covering different years and in some instances focusing on different states, counties or type of road user.

Table 2.3: Global distribution of included articles.

Continents	**Total number of studies (%)**	**Countries (number of studies)**	**Citations**
Asia	4 (9.5%)	Jordan (2) Iran (1) Hong Kong (1)	[53, 54] [67] [56]
Australia	1 (2.3 %)	Australia	[82]
Europe	19 (45.2 %)	Norway (4) Sweden (4) United Kingdom (3) Spain (2) Slovakia (1) Czech Republic (1) Portugal (1) Scotland (1) Nor, Swe, Port, Fin (2)	[40, 43, 58, 59] [51, 52, 61, 74] [57, 78, 83] [42, 84] [77] [77] [70] [60] [45, 46]
North America	16 (38.1 %)	United States (12) Canada (4)	[41, 48-50, 65, 68, 71-73, 75, 80, 81] [62, 66, 69, 79]
Oceania	1 (2.3 %)	New Zealand	[47]
South America	1 (2.3 %)	Brazil	[64]

2.5.4 Study Timeline

The year of publication (Figure 2.2) and study duration (Figure 2.3) for all included studies were wide and varied. The earliest article was published in 1974 in the United States [81] and the most recent in 2017, in Scotland [60], while the shortest and longest study periods were 10 months [68] and 18 years [70], respectively. The overall average study period was 3.8 years.

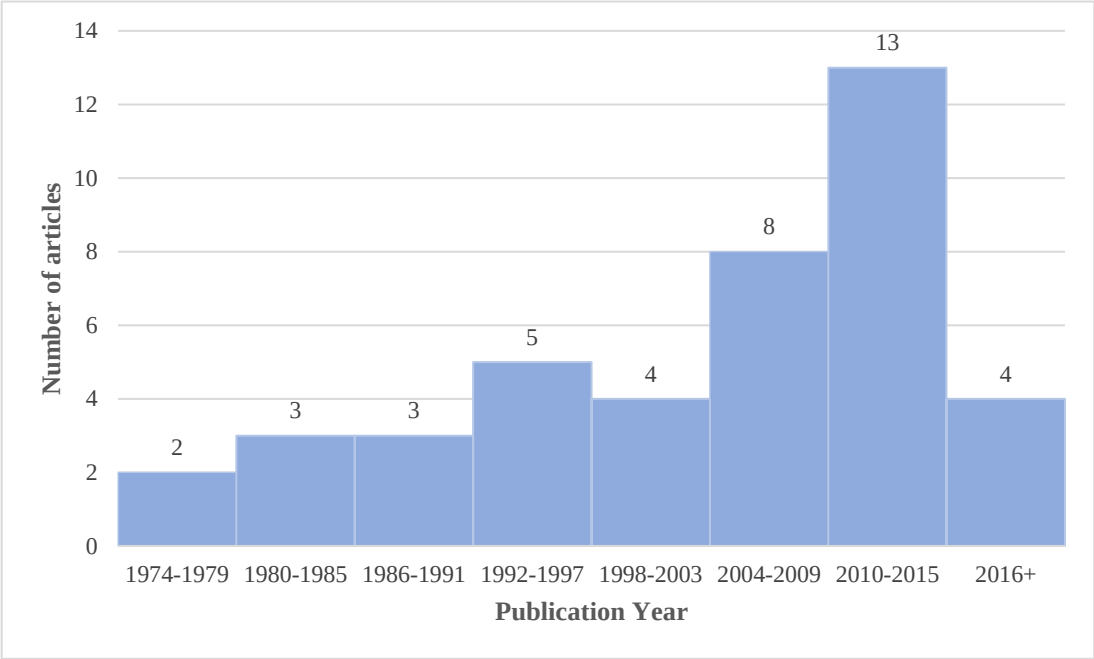


Figure 2.2: Number of included articles published in each of the "publication year" ranges.

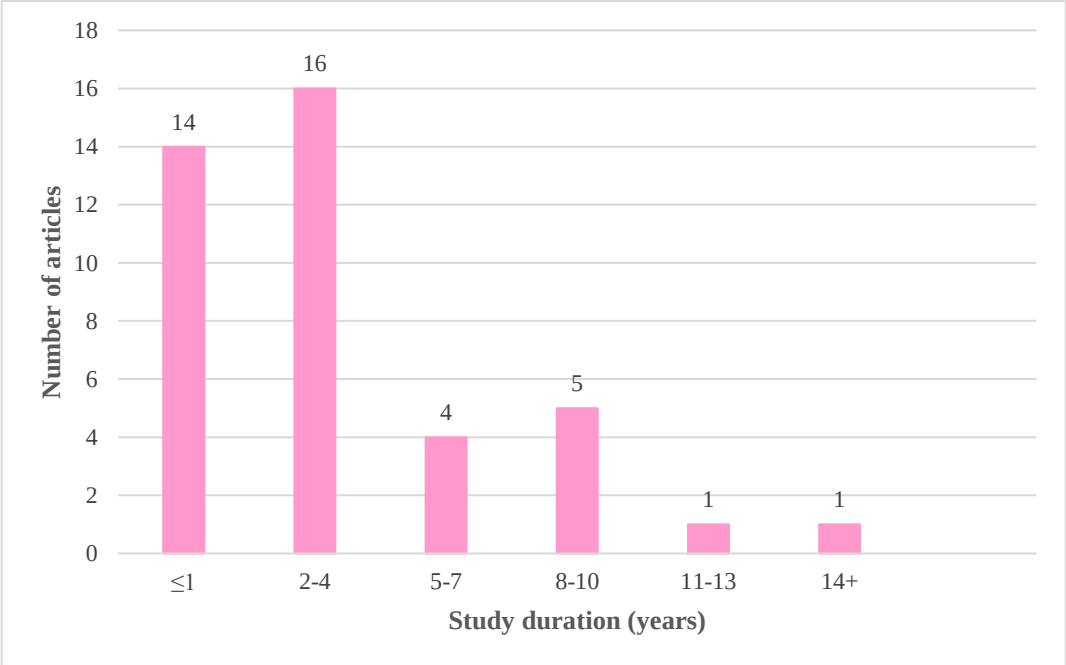


Figure 2.3: The number of articles with a study period within each of the "study duration" ranges.

2.5.5 Study Population

Road users involved in fatal RTAs were the population of interest in all 42 included studies. However, the most prominent subgroup under investigation were drivers (28 articles -66.7%) of motorised vehicles [40-42, 45-52, 56, 58, 59, 61, 62, 66-68, 71, 74, 75, 79-84]. As shown in Figure 2.4, only 5 (11%) studies comprehensively looked at all road users simultaneously i.e. drivers, passengers, pedestrians, motorcyclists, and cyclists [57, 63-65, 77].

Only 32 studies reported on distribution of the victims' sex, with male fatalities being more prevalent in the samples investigated. The mean number of males investigated in these studies was 1734 whereas that of females (only reported in 31 studies) was 503. The age of the victims reported in all the 42 studies were varied, ranging from as young as 17 years to as old as 80 years. For most studies, the highest drug prevalence was in individuals aged between 20 to 40 years old.

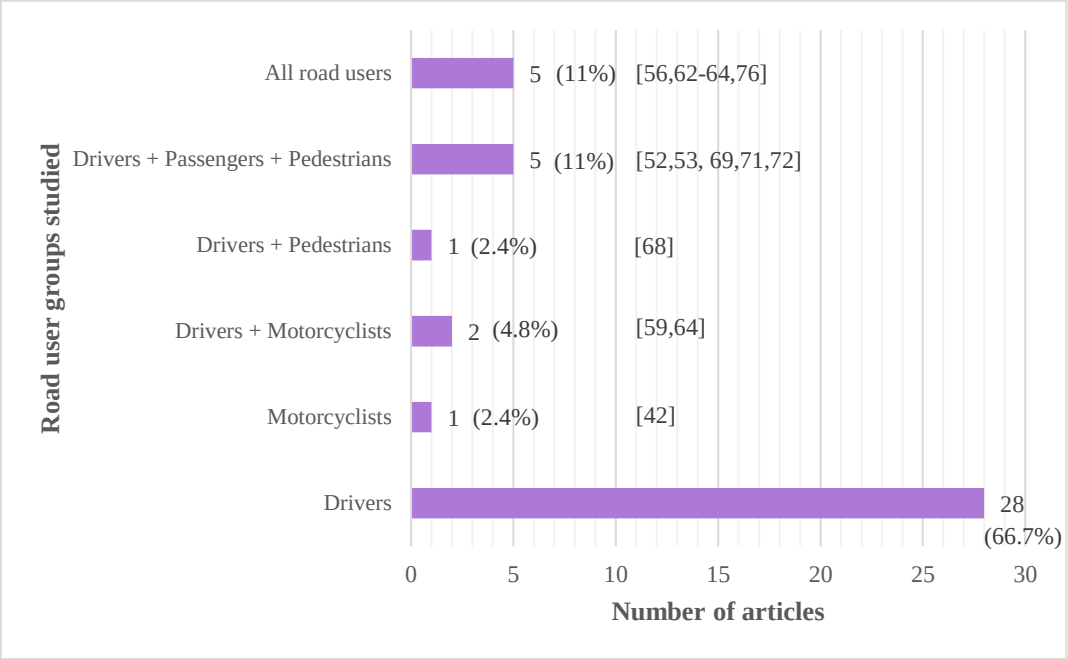


Figure 2.4: The proportions of the different road user group studied across the included articles.

2.5.6 Measurement of Outcome Variables (in the individual studies)

Of all included studies, prevalence of drugs detected was the major outcome variable measured (n=32) with half of these articles lacking significant statistical analyses such as Chi-Square tests and *p*-value reporting. Of the articles reporting statistical prevalence, 14 performed Pearson's Chi Square test, and 9 reported *p* -values in relation to the prevalence of substances detected [42, 43, 46, 50, 52, 59, 61, 63, 77]. Incidence was the next important measurement of outcome variable

(n=6) with all but one study lacking any statistics. The remaining articles reported prevalence ratios with statistical analyses reported as either p-values and/or 95% confidence intervals. These statistical analyses were not in relation to the prevalence of drugs detected but rather on the sample demographic variables or crash characteristics (single vehicle crash or multiple vehicle crash).

2.6 Outcome Measures

2.6.1 Drug Screening Methods

All included studies conducted a broad drug screening for licit and illicit substances, except for one study which screened for drugs other than alcohol [77]. Qualitative analyses were also performed in 16 studies where six only screened for substances [45, 46, 52, 53, 64, 67]. These studies only used one technique, whereas ten performed screening and confirmatory analyses [42, 54, 57, 60, 61, 63, 65, 68, 75, 78] by employing two or more different techniques for confirmation. Quantitative analyses (screening and quantification) were also performed 16 studies [40, 43, 44, 47, 50, 55, 56, 58, 59, 66, 69, 71, 73, 74, 76, 80]. There were 10 studies [41, 48, 49, 51, 62, 70, 72, 77, 79, 81] which had no toxicology analysis details.

Various techniques were used in these studies. The most common technique used for these toxicological analyses was gas chromatography mass spectrometry (GC-MS) (n=32). Headspace GC or GC-flame ionisation detection (GC-FID) were used predominately for alcohol analyses. Immunological assays, GC-MS and/or liquid chromatography mass spectrometry (LC-MS), tandem mass spectrometry (LC-MS/MS) and high performance liquid chromatography mass spectrometry (HPLC-MS) were used in 20 studies [40, 42, 50, 52, 56-61, 63, 65, 66, 68, 72, 74, 75, 78, 83, 84]. Ultraviolet spectrophotometry (UVS) [73] as well as thin layer chromatography (TLC) [81], were reported in single studies respectively. Other techniques are mentioned in Table 2.4.

2.6.2 Prevalence of Alcohol and Drugs

Reporting of the prevalence of alcohol and drugs varied in the studies in that some recorded the overall prevalence whereas others reported on the prevalence of the specific drugs/drug classes detected. The overall prevalence for both alcohol and drugs was reported in 25 studies [42, 43, 45, 46, 50-52, 58, 59, 62-65, 68-70, 72-75, 78, 80-82, 84]. On the other hand, seven studies reported alcohol only [40, 41, 47, 48, 53, 60, 71], two reported drugs only [49, 79] and eight studies did not report the overall prevalence for either alcohol or drugs in their populations [54, 56, 57, 61, 66, 67,

77, 83]. Instead, they reported on the prevalence of specific drugs/drug classes detected either in combination with alcohol or alone.

The most prevalent drugs detected in the studies were reported and these varied widely. Alcohol, however, was reported as the most commonly detected substance in 41 studies. The most prevalent drugs classes after alcohol were cannabinoids (cannabis), benzodiazepines, stimulants and opioids (Table 2.4). The common specific compounds within the first 3 classes were tetrahydrocannabinol (THC) and its metabolites, diazepam and amphetamine respectively.

Table 2.4 shows the overall reported alcohol and drug prevalence for studies from 17 countries. These were recorded as ranges for multiple studies originating from the same country. The samples tested, techniques used, analysis performed, most common drug classes and the specific compounds reported are illustrated.

Table 2.4: Samples, techniques and overall drug and alcohol prevalence per country.

Country	Overall alcohol prevalence (range)	Overall drug prevalence (range)	Sample(s)	Techniques	Analysis	Drug classes	Specific drugs
Australia	29.1%	26.7%	Blood	None mentioned	Screen and confirmation	Benzodiazepines Opioids Stimulants	
Brazil	36.1%	17.9%	Blood	Head space GC-FID GC ion trap MS	Screen	Cannabis Stimulants	- Amphetamine, cocaine
Canada	48.1-57.0%	4-26%	Blood, urine, vitreous humor (VH)	Immunoassay, head space GC-FID, GC-MS, LC-MS/MS	Screen and confirmation	Antidepressants Benzodiazepines Cannabis Opioids -	- Diazepam Tetrahydrocannabinol (THC) and carboxy THC - Salicylate
Czech Republic	34.7%	7.2%	Blood	Immunoassay (specific analytic techniques not mentioned)	Screen and confirmation	Benzodiazepines Cannabis Stimulants	
Hong Kong	-	-	Blood, urine	Immunoassay, GC-FID, GC-MS or LC-MS, GC-NPD[a], HPLC-DAD[b]	Screen and confirmation	Stimulants Cannabis Benzodiazepines Dissociative anaesthetics	Methylenedioxymethamphetamine (MDMA) - - Ketamine
Iran	-	-	Blood, VH, tissue	GC, HPLC	Screen	Opiates	
Jordan	37.1%	-	Blood, urine, VH	GC-FID, GC-MS	Screen and confirmation	Anti-depressants Barbiturates Benzodiazepines Opioids	
New Zealand	34.0%	-	Blood	Immunoassay, GC-MS, GC-NPD, LC-MS-MS	Screen and confirmation	Stimulants Benzodiazepine Opioid	Methamphetamine Diazepam Methadone
Norway	25-44.6%	15.3-16.4%	Blood	Immunoassay, GC-MS, HPLC, GC-MS/LC-MS	Screen and confirmation	Benzodiazepines Cannabis Benzodiazepines Stimulants -	Diazepam THC - Amphetamines, cocaine, methamphetamine, MDMA Methadone
Portugal	55.0%	-	Blood	GC-FID, GC-MS	Screen	Opiates Cannabinoids	
Scotland	24.0%	-	Blood	Immunoassay, GC-FID, GC-MS or LC-MS/MS	Screen and confirmation	Benzodiazepines Cannabinoids Opioids	

Slovakia	N/A	-	Retrospective study			Benzodiazepines Cannabis	
Spain	32-50.5%	9.1-63.7%	Blood	Immunoassay, head space GC-MS, GC-MS, HPLC	Screen and confirmation	Anti-depressants Analgesics Benzodiazepines Cannabis Designer drugs Opiates Stimulants	
Sweden	21-67%	7-10%	Blood, urine, VH	Immunoassay, head space GC-MS, GC-MS, LC-MS	Screen and confirmation	Analgesic Anti-depressant Benzodiazepines Cannabis Sedative hypnotic (Z-drugs) Opiates Stimulants - -	Paracetamol Citalopram Diazepam, flunitrazepam THC Zopiclone - Amphetamine, cocaine, MDMA GHB[c] Tramadol
UK	36.0%	7.4%	Blood, urine	Immunoassay, GC–FID, GC–NPD, GC–MS, HPLC–DAD	Screen and confirmation	Anticonvulsant Antihistamines Benzodiazepines Cannabis	
US	13-70%	5-81%	Blood, urine, VH, bile	Imunoassay, head space GC, GC-MS, GC-NPD, GC-FID, HPLC, UVS, TLC	Screen and confirmation	Barbiturates Benzodiazepines CNS Depressants Cannabis Stimulants Narcotic analgesics Sedative hypnotic Sympathomimetic drug -	Phenobarbital Diazepam - Cocaine, amphetamines, methamphetamine Morphine/Codeine, Propoxyphene Methaqualone Penylpropanolamine, ephedrine, pseudoephedrine Phencyclidine
Fin, Nor, Swe, Port	42-44.9%	31-48%	Blood	Head space GC, HPLC/ UPLC[d], GC MS-MS/NPD	Screen and confirmation	Benzodiazepines Cannabis Stimulants	- THC Amphetamine

a. GC-NPD: Gas Chromatography – Nitrogen Phosphorous Detector
b. HPLC- DAD: High-Performance Liquid Chromatography with Diode-Array Detection
c- GHB: Gammahydroxybuturate
d- UPLC: Ultra Performance Liquid Chromatography

The information in this table has been grouped together from the studies to illustrate alcohol and drug prevalence per country. Where there were multiple studies originating from one country, the prevalence was expressed as a range and single studies have one value (except the UK- only one study reported overall prevalence for alcohol and drugs). In some studies, only drug classes were reported without mention of the specific drugs within that class and in other instances, the specific drugs were reported and not classified into drug classes.

2.6.3 Extent of routine toxicological analysis in fatal RTAs

Routine toxicological analysis was not explicitly reported in all the studies and some studies within same country reported different information regarding the routine testing. There were ten studies in which there was no mention on routine testing [42, 45, 46, 51, 59, 66, 69, 70, 79, 84], twelve in which routine toxicology was alluded to [40, 49, 52, 57, 58, 60, 61, 67, 70, 74, 82, 83], eleven in which routine toxicology was reportedly not performed [43, 53, 54, 56, 62-64, 77, 78, 80, 81] and nine in which the testing was said to be inconsistent between the states [41, 48, 50, 65, 68, 71-73, 75] .

2.7 Discussion

2.7.1 Quality of articles

The number of articles present on this specific question are very few considering how large the global burden of RTFs is, and the acknowledgement that alcohol and drugs are major risk factors to traffic deaths. Following the review, most of the articles were deemed to be of medium to poor quality. There is a need to reiterate the importance of good quality research for this topic. It is understood however, that post-mortem research is extremely difficult due to the nature of the work as well as medico-legal and ethical requirements. Analyses regarding quality assessment were not performed here due to time restrictions. However, a meta-analysis to quantitively compare the prevalence data according to the study quality will be conducted.

2.7.2 Study Characteristics

Most of the studies originated from developed countries and only one each from Brazil, Iran and Jordan, which are considered developing countries. Six out of the seven continents were represented by the studies with most originating from European and North American populations respectively. No studies originated from Africa. This is quite interesting as it has been reported that, although RTFs are a global burden, most are contributed by low-middle income countries [85] most of which are in Africa. There have been studies published regarding RTAs and drug use separately and the effects they have on public health and the economy [86, 87]; however, the lack of resources and slow growth of forensic toxicology capacity in these countries is most likely the reason for the scarcity of literature on the specific topic of this review [88]. The years of publication for most of these studies (31%) were between 2010-2015 which are more recent years. This is most likely due to the acknowledged burden of fatal RTAs caused by impairment as well as effort towards understanding the role and involvement of drugs in these fatal RTAs.

2.7.3 Demographics

The population demographics reported indicated that males made up the dominant proportion of fatally injured road users in which drugs were detected. The age group mostly affected was 20-40 years old. These are young, working class individuals who are meant to be contributing to the economy of the countries [89]. It may be suggested that this burden is generating economic adversity due to the loss of family breadwinners [90].

The most studied road user group was drivers of motorised vehicles. This might be due to the fact this is a highly monitored victim group [57] and so most the data available is predominantly based on this group. In general, there are likely also more drivers than other road users in certain countries from which this data was reported. Very few studies investigated the presence of drugs in all road user groups and yet what has been reported in other literature, particularly from a global perspective, is the most vulnerable road user groups are pedestrians, cyclists and motorcyclists [91]. More research and monitoring of routine toxicological data from these cases, is necessary to get further insight into drug use and its contribution to fatal RTA by these vulnerable road user groups.

2.7.4 Substance analyses

An equal number of articles reported on having performed qualitative and quantitative toxicology analyses. For those reporting on the latter, quantification was done mostly for alcohol to determine the number of decedents who had BAC above the legal limits for the respective countries. The main specimen used in these studies was blood, followed by urine. Although post-mortem vitreous humor and post-mortem urine alcohol concentrations are obtained and can usually be interpreted to indicate previous ethanol administration, blood alcohol concentration is most often obtained for interpretive value and the standard technique is head space GC-FID [92]. Only a few of the studies quantified drugs other than alcohol. The effects of alcohol impairment in relation to BAC has been extensively studied [61, 70] and so specific legal limits for BAC in drivers have been defined in road traffic legislation. This is typically not the case for other drugs.

There are many challenges when it comes to interpretation of drug concentrations in biological samples, especially post-mortem samples. The specimens used for drug screening need consideration regarding the complexity of the sample, the technique to be used as well as expected results. For example, urine is simpler specimen compared to blood or VH, however, detection of substances in urine is only indicative of exposure. Quantification may not offer valuable information since concentrations are generally higher in this specimen and do not correlate to

blood levels or toxic effects [93]. Relating concentrations of drug to levels of impairment is complex and often not possible in a post-mortem context [63]. A phenomenon known as post mortem re-distribution, in which drug concentrations alter after death contribute to this uncertainty in determining the role of a drug in the victim of a fatal RTA [50, 52, 84]. In addition, both autolysis and putrefaction, which are decomposition processes may increase or decrease the levels of drugs in post mortem specimens [94]. The chemical stability of the drugs is also a factor to be considered particularly with highly volatile substances which may break down rapidly after death [95]. Furthermore, storage of samples may also affect the stability of drugs present where improperly stored samples or long storage periods may result in degradation of drug compounds [96]. The interpretations of drug post-mortem concentrations and their relation to impairment at the time of the collision, must always be done with caution, and with consideration of individual and circumstantial factors.

2.7.5 Prevalence of substances

The most prevalent licit substance detected in these studies was alcohol. This finding is of no surprise and is in keeping with numerous studies that have reported a high association of alcohol impairment in RTAs [97-100]. Driving under the influence (DUI) of alcohol exponentially increases the risk of serious accident or death [101]. Even with small amounts of alcohol consumption, drivers are twice as likely to be involved in an accident than sober drivers [102] and poly drug use, particularly with alcohol pose an even greater risk of being involved in a traffic accident [29]. Although there have been efforts worldwide to reduce drink driving through publicity, education and tough drink driving laws and penalties, drunk driving is still a significant global public health burden [103].

The most prevalent non-alcoholic drugs were cannabis, benzodiazepines, stimulants and opioids, many of which are common global drugs of abuse [104, 105] and have been observed as contributors to impairment while driving. The most prevalent specific drugs in those drug classes were THC, diazepam and amphetamines. A point worth mentioning is that, some studies only screened for drugs, i.e used one technique to detect substances in the specimens. The technique used for screening is important because some techniques are not sensitive enough to distinguish between drug classes, let alone detect specific drugs [106]. This is the case with immunoassays, in which a lot of studies used this technique for screening. As such, it is recommended that confirmatory tests be performed with a different, more specific technique to allow for unambiguous detection [107]. Another point worth mentioning is targeted versus non-targeted

screening. The former is screening for specific drugs whereas the latter is a broad-spectrum screen for presence of any drugs. The strategy chosen, will have a bearing on the drugs detected and the prevalence thereof. Although this was not mentioned explicitly in the studies, it is an aspect to keep in mind in the interpretation of the results regarding drug prevalence.

2.7.6 Routine toxicological analysis

The extent of routine toxicological analysis for fatal RTAs was not easy to determine as not all the studies explicitly mentioned or discussed this aspect. This was assessed by the reviewer based on the information in the methods and discussion sections of the studies. In addition, in some studies from the same countries, information regarding routine testing was different and so this made it difficult to determine which countries might routinely perform toxicological analyses. A possible explanation for the seemingly contradictory information is that different areas within the same country are serviced by different laboratories which may or may not have the capacity to perform toxicological analyses. However, what was noted from the studies was the valuable information provided by these analyses which would aid in the development of drug use management and interventions [77]. It's important to note that there are no universal legislative / laboratory requirements with regards to forensic toxicological testing in these cases. There are some countries which have employed different approaches, namely; i) identification of signs of impairment, ii) identification of drugs in biological specimens above a specific or cut off concentration (per se limits) or zero tolerance if per se limits have not been determined and iii) mixed systems combining the both impairment identification and per se limits [108]. There are however, recommendations made by Logan *et al.* [109] on the drugs which should be screened for in such cases. These recommendations were written in an effort to standardise toxicological analyses in such cases and are therefore, a good starting point as guidelines for countries to implement routine toxicological testing.

2.7.7 Limitations

The most notable limitations in the included studies were selection bias and non-responder bias. Most of the studies focused on the driver user group only. A small number of studies actually had all user groups as part of their study sample. In addition to that, most of the studies had a low response rate where most of the loss of cases was due to mainly: (i) lack of adequate case information leading to exclusion based on the criteria, (ii) insufficient samples for drug analyses and (iii) inappropriate or no samples collected for testing. Studying only one group of the road

user population and the high loss of eligible cases is not representative of the road user population and potentially leads to bias in the data where there might be an under or over representation in the estimates reported [41, 43, 58, 70, 82].

Another limitation of the studies in general was is a lack of global consistency in toxicological analyses and reporting. Because of this, there is bias in the results- for example, the studies which screened only urine for drugs would identify more drugs than those that screened blood. Likewise, studies which performed a targeted screen may identify fewer drugs than a non-targeted screen. Another point of consideration is that the limits of detection and quantitation of analytes vary according to sensitivity of instruments. Collection procedures and sample storage could also result in biased findings.

Additionally, neither of the studies had control groups (non-fatal DUI of drugs cases) in which to compare their findings. This comparison would provide better information of drug trends and the prevalence thereof. The role of these drugs in death was also not assessed as this is a complex and multi-factorial process often requiring case specific evaluation and determination.

The limitations of this review itself is the potential reviewer bias as the reviewer independently screened the articles and extracted the data with only a 7% random check by two other reviewers. Secondly, this review was based on reports from higher-income countries and none from lower income countries as none were available or fit inclusion criteria. This potential bias was addressed as best as possible through extensive searching of grey literature for unpublished material, reports and conference papers. Lastly, the exclusion of eligible articles due to lack of access to the full texts. An email was sent to the relevant authors but, unfortunately, there has been no response to date.

2.8 Conclusion

Studies investigating the prevalence of substances in road users of fatal RTAs originated mostly from developed countries, which typically have the resources and capacity to carry out such research and routine services. This has provided meaningful information and has highlighted that alcohol, cannabis, stimulants, benzodiazepines and opioids are more so reportedly involved in fatal RTAs and there remains a clear need to intensify strategies to prevent drunken and drugged driving. The challenges regarding drug use and traffic accidents are complex and multifaceted, and more research is necessary to understand and purport the role of drugs and their concentrations in post-mortem samples to causing and contributing to fatal RTAs.

This review also highlighted a gap in the literature regarding drug use in the lesser studied vulnerable road user groups. Further, research and routine reporting is needed to gain a more in-depth insight into the involvement of drugs in these vulnerable groups. Although routine toxicological testing for fatal RTAs was not highly evident in this review, what was noted was toxicological analyses in these fatal RTA cases provide insightful information regarding the trends of drugs and alcohol in victims of fatal road collisions.

Developing research and routine toxicological service capacity within LMICs is necessary to investigate these trends further in countries seen as providing a higher burden of RTFs. Developing consistency in testing and reporting on a national and international level, would be worth striving towards to better understand this burden as a whole.

Chapter 3: Toxicological Findings in Fatal Road Traffic Accidents in Cape Town: Introduction

Chapter 1 highlighted the global burden experienced due to RTFs and the risk factors that contribute to these fatalities. The particular risk factor focused on was driving impairment due to presence of alcohol and drugs of use and abuse. The prevalence of substances in global RTF was investigated systematically in Chapter 2, which included evaluation of the role and extent of toxicological analyses used in these fatalities.

It was necessary to compare the findings outlined in Chapter 2 to a local context within SA. The following chapters discuss traffic deaths in Africa, with a focus on Cape Town, Western Cape, where the prospective study was performed. Insight into the drugs of abuse in SA is also provided to contextualise the study rationale.

3.1 Distribution of Road Traffic Deaths

3.1.1 Distribution as per WHO's world group regions

Geographical differences in road traffic death rates exist both across the six WHO regions (Figure 3.1) and within those regions themselves [13, 110]. Countries in Africa and South-East Asia report traffic death rates much higher than the global rate and the developed Europe and Americas regions (Figure 3.1) [110].

Figure 3.1: Rates of road traffic death per 100,000 population by WHO-defined regions, 2016 [2].

3.1.2 Distribution as per country income classifications

LMICs produce approximately 90% of the global road traffic deaths, accompanied by increased urbanization and an increased number of vehicles on the road [85]. LMICs in the Africa region have reported rates of 23.6 per 100 000 and 29.3 per 100,000 population, respectively [110]. RTAs affect vulnerable road users i.e. cyclists and pedestrians [91], which is particularly the case in LMICs where these groups are highly inter-mixed on the roads [13]. Contrary to LMICs, there has been a decrease in the rates of RTA fatalities over time in high income countries (HICs), but still, the global economic costs is rising substantially [111]. A global estimate of US $518 billion annually is lost due to RTAs in the form of emergency services costs, medical costs, legal and court cost, lost productivity, property/motor vehicle damages, workplaces loss, insurance administrations [112] . LMICs account for an estimated US $65 billion, which is more than these countries usually receive in financial support [113, 114]. These observations illustrate the fact that LMICs carry a large portion of the burden of the world's road traffic deaths.

3.2 The Burden of Road Traffic Deaths in South Africa

With LMICs in Africa contributing the highest rates of global road traffic morbidity and mortality, a closer look at the traffic death rate in SA is necessary to gain insight into the local context.

In 2007, road traffic injuries in SA were ranked second highest after interpersonal violence causing death [115]. In 2017, 14 050 RTFs were reported, with pedestrians and passengers being most affected (Figure 3.2) [116]. Half of the deaths affected people aged between 20-44 years in all road user groups [117, 118]. In 2015, the cost of RTAs was reported to be R142.95 billion, approximately 3.4% of the country's gross domestic product (GDP), whereas in other similar countries, the average cost was 2.2% of their GDP [119, 120].

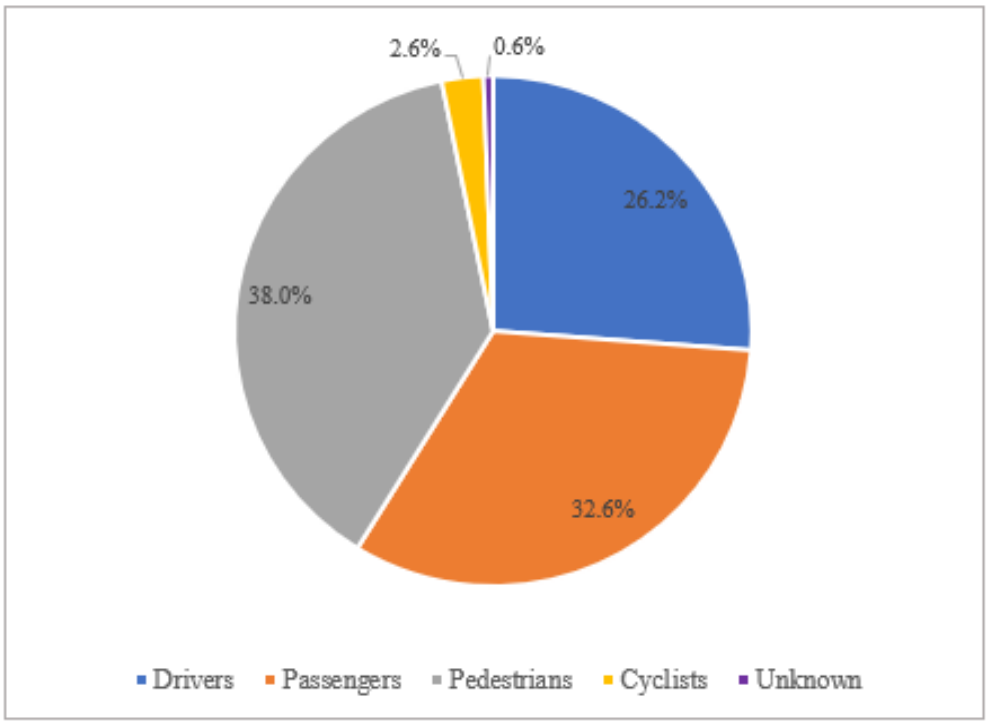


Figure 3.2: Percentage distribution of fatalities per road user group in 2017 in SA [9].

The traffic fatality rate of SA in 2016 was 25.9 per 100 000 [110], one of the highest rates in the Africa region. Based on the Road Traffic Management Corporation's (RTMC) 2017 report, four out of the nine provinces in SA – Gauteng (GA), Kwazulu Natal (KZN), North West (NW) and Western Cape (WC) have achieved greater fatality reductions than the other five provinces [116]. Although there has been a reduction in traffic fatalities in the WC province, the fatality rate of Cape Town is 30 per 100 000 [121] which is higher than that of Africa region itself.

3.3 Drugs of Abuse in South Africa

The information available on the extent of drug use and drug-related deaths in LMICs is lacking due to the limited resources and expertise to evaluate the issue [122]. SA is no exception to this.

Although there exists an alcohol or drug (AOD) surveillance system known as South African Community Epidemiological Network on Drug Use (SACENDU), the data is based on reports of patients admitted into AOD treatment facilities [123] and is therefore not representative of the general population. Thus, determining the prevalence of drug use in SA is challenging due to the lack of recent nationwide drug use surveys [124]. Toxicological analyses in all unnatural deaths is also not performed routinely, thus monitoring the drugs present in the deceased populations in SA is not currently feasible.

Information gathered from the limited research suggests that alcohol is the most widely abused substance [125] with cannabis, methamphetamine, heroin and cocaine being the main illicit drugs of abuse in SA [126, 127]. Cape Town, the capital city of Western Cape province has been noted as having high levels of drug abuse and drug related problems [123]. There is a continual increased use of methamphetamine, locally known as "tik," which has become the major drug of abuse in the province, [128] followed by cannabis [129] and methaqualone ("mandrax").

While cognitive, motor and behavioral impairment associated with psychoactive drug use is recognised, there is currently a lack of research in SA investigating the prevalence and role of drugs in RTA fatalities.

3.4 The South African Medicolegal System

In SA, the Inquest Act (Act 58 of 1959) provides for procedures to be followed in cases of death which are due to unnatural causes (i.e. suicide, homicide, procedure-related and accidents) [130]. Certified Forensic pathologists in provincial Forensic Pathology Services (FPS), perform post-mortem examinations on suspected unnatural cases to determine the COD. The manner of death (e.g. suicide, homicide, accident) is usually determined by the Court during inquest or criminal proceedings. At the time of performing the autopsy, pathologists will assess the need to request drug and/or alcohol testing based on autopsy findings or any relevant/suggestive history that may be available at the time [131].

There are currently four National Health Forensic Chemistry Laboratories (FCL) in SA, which perform analyses on biological specimens (e.g. blood) in driving under the influence (DUI) and post-mortem cases. In RTAs, usually only tests for blood alcohol concentration are routinely performed in DUI cases.

In post mortem cases (includes drivers, passengers, pedestrians, motorcyclists and cyclists) the pathologist will use their discretion to collect biological specimens for volatiles and toxicological analysis based on case history and information provided. However, it is historically common practice amongst the pathologists to collect blood for alcohol analysis only. This is because there is too large of a forensic back log to be able to test these cases for other drugs as well. In post-mortem RTF cases, as the COD is known (e.g. trauma), which is the primary mandate of the pathologists, many will choose not to submit specimens for additional drug testing.

Cape Town houses one of the four FCLs, that also performs tests for Northern and Eastern Cape. City of Cape Town forming the metropolitan municipality has an estimated population of approximately 3.7 million. and is divided into East and West. In WC, there are seventeen FPS facilities across the province with different intake numbers based on their location. [132]. The two largest mortuaries are academic and admit an average case load of 4000 suspected unnatural deaths per annum. One of these is Salt River Mortuary (SRM), which is associated with University of Cape Town (UCT) and covers the West Metropole of Cape Town [133].

3.5 Research Rationale

There are an increasing number of studies investigating presence of substances (alcohol and other drugs) in RTFs. Alcohol is the most investigated substance and its effects on impairment are well understood. The involvement of other drugs of use and abuse must not be disregarded as their role in these fatalities is still not yet fully understood.

In SA, fatal RTAs continue to be a common occurrence, contributing to the burden of violence and injury/trauma nationwide. Little is known on the role of substances in road traffic deaths locally due to limited research with some information available from organisational reports (e.g. WHO, South African Police Services, Road Traffic Management Centre). This information though, is at times, outdated and inaccurate due to underreporting, resulting in a knowledge gap. The lack of recent data pertaining to licit of illicit drug use is a challenge in SA, particularly in the forensic context where national medicolegal standards on the toxicological investigation of road traffic deaths are lacking.

Internationally however, updated recommendations for toxicological investigation of motor vehicle fatalities were recently published [109], speaking to three tiers of drugs and drug classes which should be screened for in fatal RTAs. The intention of these recommendations is the

standardisation of toxicological practices and improvement of the quality of data obtained from such cases.

Given that international studies have reported on the presence and role of drugs in fatal RTAs, that there are international recommendations on the toxicological testing of such cases, and finally that there are high rates of RTAs and drug abuse in SA; it is apparent that there is a need to assess the current SA forensic toxicology practices and to interrogate drug use and patterns in fatal RTAs. This study was a pilot project undertaking to begin to investigate drugs in RTA cases in the West Metropole in Cape Town, so as to contribute to understanding this burden and identify avenues for further assessment in research and service in future.

3.6. Aim and Objectives

This study aimed to conduct a pilot toxicological investigation in a cohort of road traffic fatality deaths from SRM in Cape Town. The objectives were to:

i. Investigate the presence of substances (other than alcohol) in a cohort of road deaths through toxicological screening in post-mortem biological specimens for drugs of use and abuse using liquid chromatography quadrupole time-of-flight mass spectrometry (LC/QTOF-MS).
ii. Assess any drug trends/patterns, demographics and case characteristics identified through basic descriptive analysis of the RTA cohort.
iii. Assess the applicability and utility of this type of toxicological data in road traffic research, and the relevance of routine toxicological investigations in these cases.

Chapter 4: Materials and Methods

This chapter introduces the population under investigation in this preliminary prospective study and delves into the setting of the study, the analytical and statistical methods used, along with the ethical considerations involved within research of post-mortem cases.

4.1 Study Population

The population of interest for this study included all road users (drivers and passengers of any motor vehicle, pedestrians, motorcyclists and cyclists) involved in fatal traffic collisions, who were admitted to SRM between 6 June to 28 September 2018.

A brief review of the SRM's internal autopsy database (HREC:270/2018) (Appendix F) of cases admitted between 01 January 2016 and 31 December 2017, revealed that on average, there were 344 (13% of total admissions to SRM) RTA cases annually of which only 5 (1.5%) on average had toxicology analysis requested. Following this review, an average of 113 RTA cases were identified in the four-month period (June-September) over the 2 years reviewed.

Given that such a prospective study has not been conducted locally before and that statistics on post mortem case counts are not available, power calculations weren't performed. The sample size estimated for this pilot study was thus 113 cases.

4.2 Inclusion and Exclusion Criteria

All cases of unnatural death other than RTAs, namely suicides, homicides, accidental and undetermined deaths not involving road users and/or vehicles, as well as cases in which no specimens (blood, urine/bile and vitreous humor) could be collected due to severe trauma were excluded (Figure 5.1). Cases in which only one of aforementioned specimens (excluding hair) could be collected were included. RTA cases in which hospitalisation and subsequent death occurred in a greater than 24-hour time frame were excluded. Decomposed and burned bodies in which samples could not be collected were omitted from the study. Children, aged younger than 10 years old were excluded since they were unlikely to represent the general population's drug use patterns. Of the 110 cases admitted, only 84 cases were eligible for the study based on the inclusion criteria.

4.3 Informed Consent in the Forensic Setting

Written informed consent was obtained from the next-of-kin of each decedent included in the study. For each eligible case, a meeting between a next-of-kin of the deceased and the researcher was arranged with the assistance of the mortuary staff. This meeting served to provide the next of kin with information regarding the study, its purpose and benefits (Appendix G) and to formally request informed consent to collect specimens from the deceased to be used in the study. An informed consent form (Appendix H) was signed by the next of kin had consent been granted.

4.4 Sample Collection and Storage

For the cases in which informed consent was obtained; femoral blood, VH, urine (or bile if urine was unavailable) and hair were collected during autopsy. Blood samples were collected in 4 ml grey top tubes (Greiner Bio-One, Kremsmünster, Austria) with sodium fluoride and potassium oxalate. VH was collected in 4 ml plain tubes (Disera, Izmir, Turkey) without additives and urine/bile in 15 ml sterile centrifuge tubes (Nest Biotechnology, Jiangsu, China). These samples were stored in the Biomedical Forensic Science Laboratory at UCT at 4^{o}C until sample analysis. Hair was collected by cutting as close to the scalp as possible to get the root ends, placed in paper and secured using a druggist fold, which was placed in an envelope and stored at room temperature [93].

4.5 Qualitative Toxicological Analysis

4.5.1 Sample preparation

Samples were prepared using a modified acetonitrile protein precipitation method [134]. For blood, VH and bile, 100 µl of the sample was aliquoted into 600 µl of acetonitrile (Merck, Darmstadt, Germany). With urine samples, 200 µl was aliquoted into 100 µl of acetonitrile. These were vortexed for ~ 5 seconds and centrifuged at 13 000 rpm for 5 minutes. The 1.5 ml screw neck glass vials (Macherey-Nagel GmbH & Co. KG, Düren, Germany) for blood, VH and bile had 0.3 ml/ 6 x 31 mm wide opening, flat bottom glass inserts (Macherey-Nagel GmbH & Co. KG, Düren, Germany) with 200 µl of the respective sample supernatant transferred into them. The vials for urine also had 200 µl of the supernatant with 800 µl of ultra-pure water added to them. All the vials were centrifuged for ~ 5 seconds before being analysed. While using an internal standard for quantitative and qualitative studies is recommended, for the purpose of screening, it was deemed

sufficient to include in the controls. Hair was not analysed in this study due to time constraints but was stored at room temperature in the Biomedical Forensic Laboratory at UCT for future analyses.

4.5.2 Screening for drugs of abuse

The analyses were conducted at the UCT Division of Pharmacology, Groote Schuur. LC/QTOF-MS analyses were performed on the ExionLC AC system (Sciex, Massachusetts, USA) coupled to a Sciex X500R QTOF-MS system (Sciex, Massachusetts, USA). Chromatographic separation was achieved by a phenyl hexyl Kinetex C18 column (50 mm x 4.6 mm, ID 2.6 µm) (Phenomenex, California, USA) at 40^{o}C with 10 mM ammonium formate (Sigma-Aldrich, Deisenhofen, Germany) used as the aqueous phase (Mobile Phase A - MPA) and 0.05% formic acid in methanol (Merck, Darmstadt, Germany) as the organic phase (Mobile Phase B – MPB). The system was run in a linear gradient from 2% to 98% organic phase (MPB) over 12 min. The total run time was 12.5 min, run at a constant flow rate 0.5 ml/min. The total volume of sample injected was 10 µl. The mass spectrometer ionization was performed in positive electron spray ionisation (ESI) mode. The ion spray voltage was 5000 V and source temperature was 500^{o}C.

4.5.3 MS Data Processing

The samples were analysed using a vSWATH^{TM} method, which is a semi-targeted screening for 725 drugs in a single run, which included drugs of abuse, over the counter (OTC) drugs, prescription drugs and endogenous compounds. The limit of detection for the method was 20 ng/ml. Data acquisition and processing was employed using SCIEX OS software version 1.4. Positive detection relied on an expected retention time within ±0.5 minutes, a mass error confidence of ±2 ppm and a library hit score of 80% minimum. Each peak was also visually verified by the operator by analysing the accurate mass details related to the mass spectrum.

4.5.4 Quality Controls

There were three quality controls used: Restek system suitability test mixture (Restek Corp, Bellefonte, PA, USA) containing amiodarone (10 µg/ml), amphetamine (10 µg/ml), caffeine (10 µg/ml), codeine (10 µg/ml), diazepam (10 µg/ml), doxepin (10 µg/ml), haloperidol (1 µg/ml) and morphine (10 µg/ml). The second control was an in-house control containing drugs of abuse, prescription and over-the-counter compounds and the third, ultra-pure water as the negative control. These quality controls were analysed with every batch of the specimens and the acceptance criteria were the same as that for the positive detection of drugs in the specimens (section 4.5.3).

4.6 Data Management

Case related information was collected regarding the date, time, area of death, scene information and the suspected manner and determined COD. The demographics of the decedent including age, sex and road user group were also recorded from South African Police Service (SAPS) and FPS documentation, and autopsy reports for each case. The signed consent forms were attached to the corresponding toxicological request forms containing the above-mentioned information for each case. The compiled forms were filed and kept in an access-controlled office in the Division of Forensic Medicine and Toxicology (UCT). To protect the sensitivity of the information collected, no identifying features of the deceased were stored, and all cases were assigned unique RTA study numbers to maintain confidentiality and anonymity. All digital case information and databases pertaining to the research referred to cases using the assigned RTA study numbers and were stored in a USB which could only be retrieved and used from the Division of Forensic Medicine and Toxicology.

4.7 Statistical analysis

Statistical analysis of the data was performed on STATA 13 [135]. Counts and proportions were tabulated for the demographic characteristics, COD and toxicological findings. For prevalence proportions of the detected drug groups, 95% confidence intervals were calculated. For comparisons between proportions, Fisher exact test was carried out where a p-value < 0.05 was regarded as statistically significant. In this study, comparisons were done between the demographic variables of the study cohort for which consent was obtained and the overall RTA cohort within the 4-month specimen collection period.

4.8 Ethical approval

Ethical approval for this study was granted by the University of Cape Town's Human and Ethical Research Committee (HREC Ref: 270/2018). All the information used in this study was kept anonymous and confidential throughout the duration of study. The study was conducted in line with the updated Helsinki Declaration (2013). Data was obtained for the comparison of included and excluded cases in the study period, from the internal autopsy database, for which ethical approval has been obtained (HREC Ref:270/2018).

Chapter 5: Results

5.1 Cohort Selection

A total of 110 RTA cases were admitted into SRM during the study period. Of these, only 84 cases were eligible for inclusion. In 54 eligible cases, informed consent was not obtained, therefore, the final study cohort included 30 cases (Appendix I) that underwent qualitative toxicological analysis (Figure 5.1).

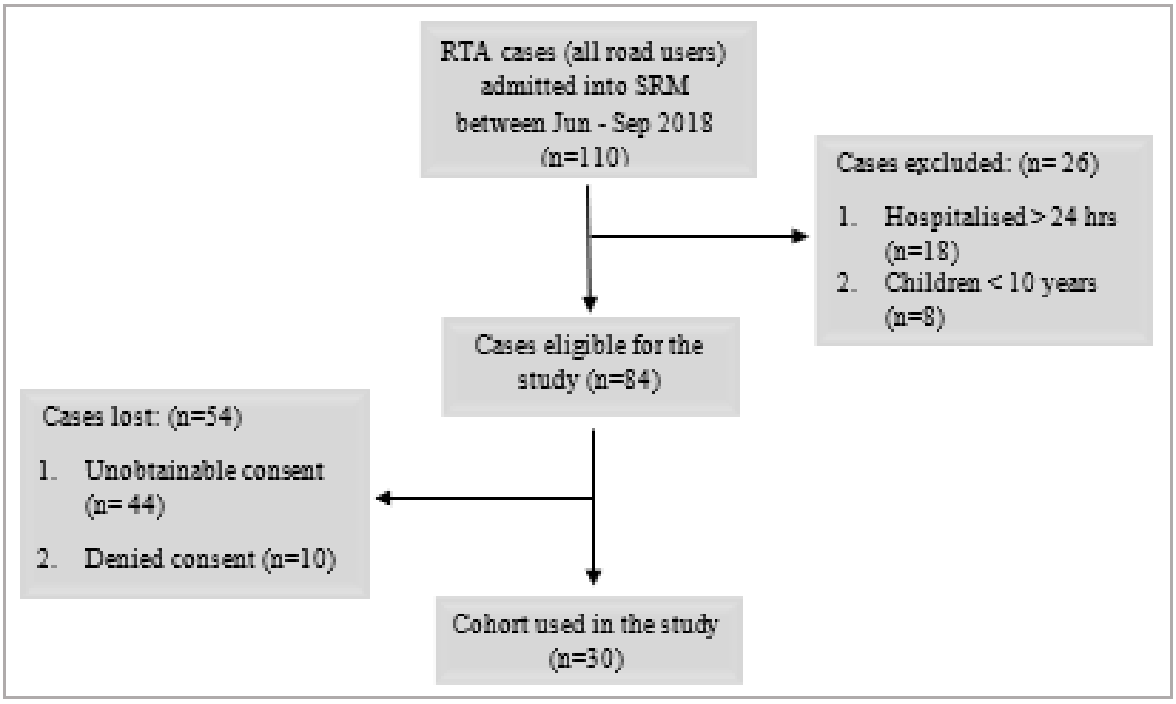


Figure 5.1: Flow diagram of fatal RTA case selection detailing the cases admitted in the study period and the reasons for exclusion and cases in which consent was not obtained.

Unobtainable consent: Researcher was unable to meet with the family due to not being notified by the forensic pathology officers.
****Denied consent: the family refused to partake in the study.***

5.2 Case Characteristics

Of the 30 cases, the median age was 39 years old (mean: 40 years, range: 61years). The proportion of males (90%) was higher than that of females (10%) and pedestrians (33.3%) dominated the cohort (Table 5.1.). The most common COD was multiple blunt force injuries to the body (43.3%). The demographics of all the RTA cases admitted into SRM in the 4-month sample collection period are also shown in Table 5.1. The 30-case cohort and the overall RTA cases are compared

in terms of their proportions and there were no significant differences observed between the two except for the pedestrian proportions in which there was a significant difference.

Table 5.1: Demographic characteristics of the 30 cases and overall 110 RTA cases admitted in the 4-month period.

	Cohort of 30 cases		Overall RTA cases		30 cohort vs overall RTA cases
	n	% of total no. of cases	n	% of total no. of cases	p-value
Age					
Median age (years)	39.0	-	35.0	-	-
Sex					
Male	27	90.0	97	88.2	0.7795
Female	3	10.0	13	11.8	0.7795
Road user group					
Driver	6	20.0	19	17.3	0.7263
Passenger	7	23.3	15	13.6	0.1971
Pedestrian	10	33.3	60	54.5	0.0394*
Motorcyclist	6	20.0	12	10.9	0.1868
Cyclist	1	3.3	2	1.8	0.6101
Unknown	-	-	2	1.8	-
Cause of death					
Blunt force trauma - head	7	23.3	31	28.2	0.5961
Blunt force trauma - neck	1	3.3	4	3.6	0.9362
Blunt force trauma - chest	2	6.7	7	6.4	0.9522
Blunt force trauma - head and chest	3	10.0	9	8.2	0.7490
Blunt force trauma - head and pelvis	1	3.3	2	1.8	0.6101
Blunt force trauma - chest and abdomen	2	6.7	2	1.8	0.1585
Multiple blunt force injuries	13	43.3	49	44.5	0.9045
Traumatic asphyxia	1	3.3	1	0.9	0.3222
Blunt force trauma - head and neck	-	-	3	2.7	-
Blunt force trauma -chest and neck	-	-	1	0.9	-
Under investigation	-	-	1	0.9	-
Crash Type					
Single vehicle collision	15	50.0	-	-	-
Multiple vehicle collision	6	20.0	-	-	-
Suburb					
Athlone	1	3.3	-	-	-
Atlantis	3	10.0	-	-	-
Kirstenhof	1	3.3	-	-	-
Lansdowne	1	3.3	-	-	-
Lentegeur	1	3.3	-	-	-
Malmesbury	1	3.3	-	-	-
Maitland	2	6.7	-	-	-
Mannenburg	1	3.3	-	-	-
Melkbosstrand	1	3.3	-	-	-
Mitchells Plain	2	6.7	-	-	-
Muizenburg	1	3.3	-	-	-
Nyanga	7	23.3	-	-	-
Phillipi	6	20.0	-	-	-
Rondebosch	1	3.3	-	-	-
Woodstock	1	3.3	-	-	-

*statistically significant

In the 30 cases, the highest proportion of males killed were in the 31-40 years age group and females in the 41-50 years. Drivers were most represented in the 21-30- and 41-50-years age groups, passengers in the 41-60 years, pedestrians in the 31-40 years age group and the proportion of motorcyclist was highest in 21-40 years age range (Table 5.2).

Table 5.2: Break down of victims by age, sex and road user group.

		n (% proportion by age bracket)						
Age range (years)	**No. of cases**	**Male**	**Female**	**Driver**	**Passenger**	**Pedestrian**	**Motorcyclist**	**Cyclist**
≤20	1	1 (100)	-	1 (100)	-	-	-	-
21-30	7	7 (100)	-	2 (28.6)	1 (14.3)	2 (28.6)	2 (28.6)	-
31-40	9	9 (100)	-	1 (11.1)	1 (11.1)	4 (44.4)	2 (22.2)	1 (11.1)
41-50	7	5 (71.4)	2 (28.6)	2 (28.6)	2 (28.6)	2 (28.6)	1 (14.3)	-
51-60	4	100	-	1 (25.0)	2 (50.0)	-	1 (25.0)	-
61-70	1	-	1 (100)	-	1 (100)	-	-	-
70+	1	1 (100)	-	-	-	1 (100)	-	-

5.3 Post mortem drug prevalence

The various specimens collected for all 30 cases underwent a semi-targeted screen and a total of 29 substances were detected and categorised into drug groups as shown in Table 5.3.

Table 5.3: Detected substances categorised into drug groups.

Drug group	Specific substance detected
Analgesics	Acetaminophen
Antidepressants	Amitriptyline
Antihistamines	Chlorpheniramine, Diphenhydramine, Doxylamine, Orphenadrine
Atypical antipsychotics	9-Hydroxyrisperidone
CNS stimulant	Amphetamine, Caffeine, Methamphetamine
Opioidss/opiates	Codeine, Codeine glucuronide, Hydromorphone, Morphine
Parasympathetic stimulant	Cotinine, Nicotine
Other prescription/OTC drugs	Chinine, Gliclazide, Ketamine and Norketamine, Lidocaine, Nicotinamide, Theophylline, Tranexamic acid, Quinidine, Quinine, Warfarin
Sedative-hypnotics	Methaqulone, Zopiclone

The prevalence of each drug group as is represented in Figure 5.2 with the three most prevalent drug groups being CNS stimulants (20 cases), other prescription/OTC drugs (20 cases) and parasympathetic stimulants (9 cases).

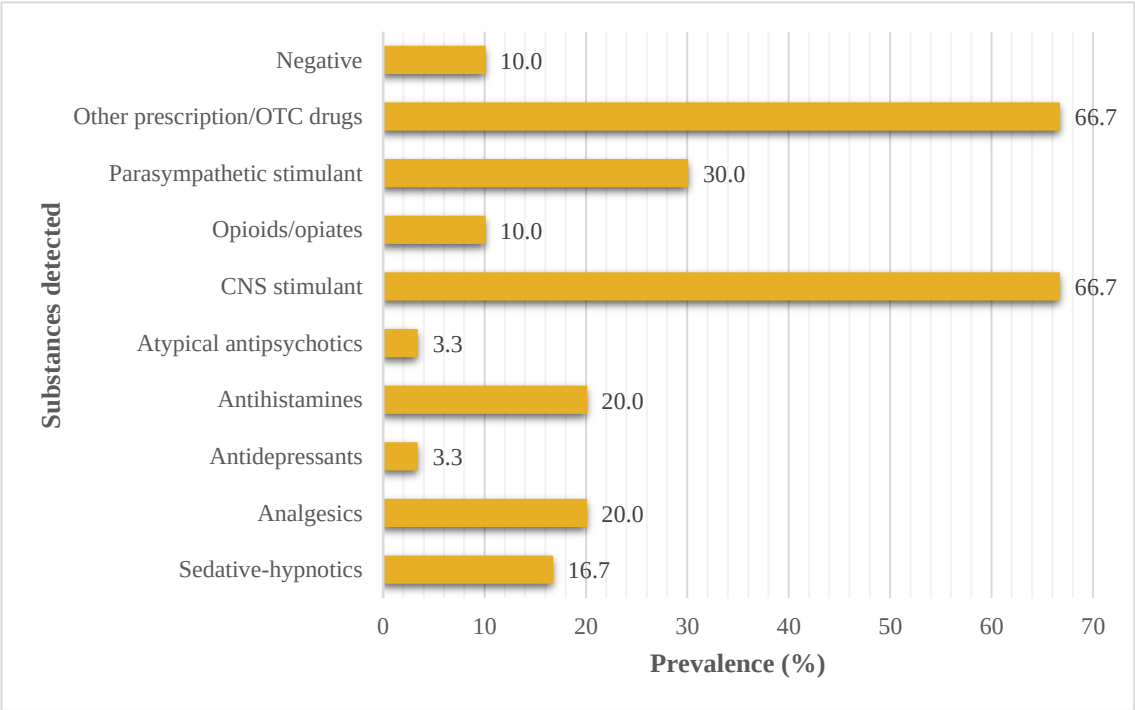


Figure 5.2: Prevalence (as a percentage) of drug groups detected in the 30 cases.

5.4 Specific drug detections in each road user group

Drugs (other than alcohol) were detected in in 27 (90%) of the cases, of which 26 (96%) had multiple substances present (Appendix I). The specific drugs detected per road user group are shown in Table 5.4. The most prevalent legal substances were caffeine (n=20) and nicotinamide (n=16). These substances were detected in all the road user groups except the single cyclist case. The most common illegal substances detected were methaqualone (n=3) and methamphetamine (n=2) which were detected in pedestrians only. The most frequent substance detections for drivers were antihistamines and other prescription/OTC drugs whereas pedestrians and motorcyclists were frequently positive for the other prescription/OTC drugs.

Table 5.4: Specific drug substances detected in each road user group.

		n (% proportion of total no. of cases)					
Drug group	**No. of cases**	**Driver**	**Passenger**	**Pedestrian**	**Motorcyclist**	**Cyclist**	**95% CI**
Negative	**3**	-	2 (6.7)	-	1 (3.3)	-	**0.026 - 0.276**
Analgesics	**6**						**0.084 - 0.391**
Acetaminophen		2 (6.7)	3 (10.0)	-	1 (3.3)	-	
Antidepressants	**1**						**0.002 - 0.191**
Amitriptyline		-	-	1 (3.3)	-	-	
Antihistamines	**6**						**0.084 - 0.391**
Chlorpheniramine		2 (6.7)	1 (3.3)	-	1 (3.3)	-	
Diphenhydramine		2 (6.7)	-	1 (3.3)	-	-	
Doxylamine		1 (3.3)	-	-	-	-	
Orphenadrine		1 (3.3)	-	-	-	-	
Atypical antipsychotics	**1**						**0.0017 - 0.1905**
9-Hydroxyrisperidone		-	-	1 (3.3)	-	-	
CNS stimulant	**20**						**0.471 - 0.820**
Amphetamine		-	-	1 (3.3)	-	-	
Caffeine		4 (13.3)	5 (16.7)	7 (23.3)	4 (13.3)	-	
Methamphetamine				2 (6.7)			
Opioids/opiates	**4**						**0.0434 - 0.316**
Codeine		1 (3.3)	-	-	-	-	
Codeine glucuronide		1 (3.3)	1 (3.3)	-	-	-	
Hydromorphone		-	-	-	1 (3.3)	1 (3.3)	
Morphine		-	-	-	1 (3.3)	1 (3.3)	
Parasympathetic stimulant	**10**						**0.179 - 0.529**
Cotinine		1 (3.3)	1 (3.3)	5 (16.7)	2 (6.7)	-	
Nicotine		2 (6.7)	-	3 (10.0)	1 (3.3)	-	
Other prescription/OTC drugs	**20**						**0.471 - 0.820**
Chinine		-	-	1 (3.3)	-	-	
Gliclazide		-	-	1 (3.3)	1 (3.3)	-	
Ketamine		1 (3.3)	-	-	1 (3.3)	-	
Lidocaine		1 (3.3)	-	-	1 (3.3)	-	
Nicotinamide		4 (13.3)	2 (6.7)	6 (20.0)	4 (13.3)	-	
Norketamine		-	-	-	1 (3.3)	-	
Theophylline		-	-	-	1 (3.3)	-	
Tranexamic acid		-	-	-	1 (3.3)	-	
Quinidine		-	-	1 (3.3)	-	-	
Quinine		-	-	2 (6.7)	-	-	
Warfarin		-	-	-	1 (3.3)	-	
Sedative-hypnotics	**5**						**0.063 - 0.355**
Methaqulone		1 (3.3)	-	3 (10.0)	-	-	
Zopiclone		-	-	-	1 (3.3)	-	

5.5 Road user groups, medico-legal findings and collision types

The most common COD among all road user groups was multiple blunt force injuries to the body and the second most common COD was blunt force trauma to the head. Drivers and motorcyclists' COD were mainly blunt force trauma to head and the body, passengers had trauma injuries to the chest and to the body and pedestrians mostly had head and chest and body blunt trauma injuries (Fig 5.3A). Single vehicle collisions (SVC) accounted for 15 (50%) of all the cases and multiple vehicle collisions (MVC) 6 (20%) cases. Pedestrians and motorcyclists were mostly involved in SVC whereas drivers were slightly more involved in MVCs (Fig 5.3B).

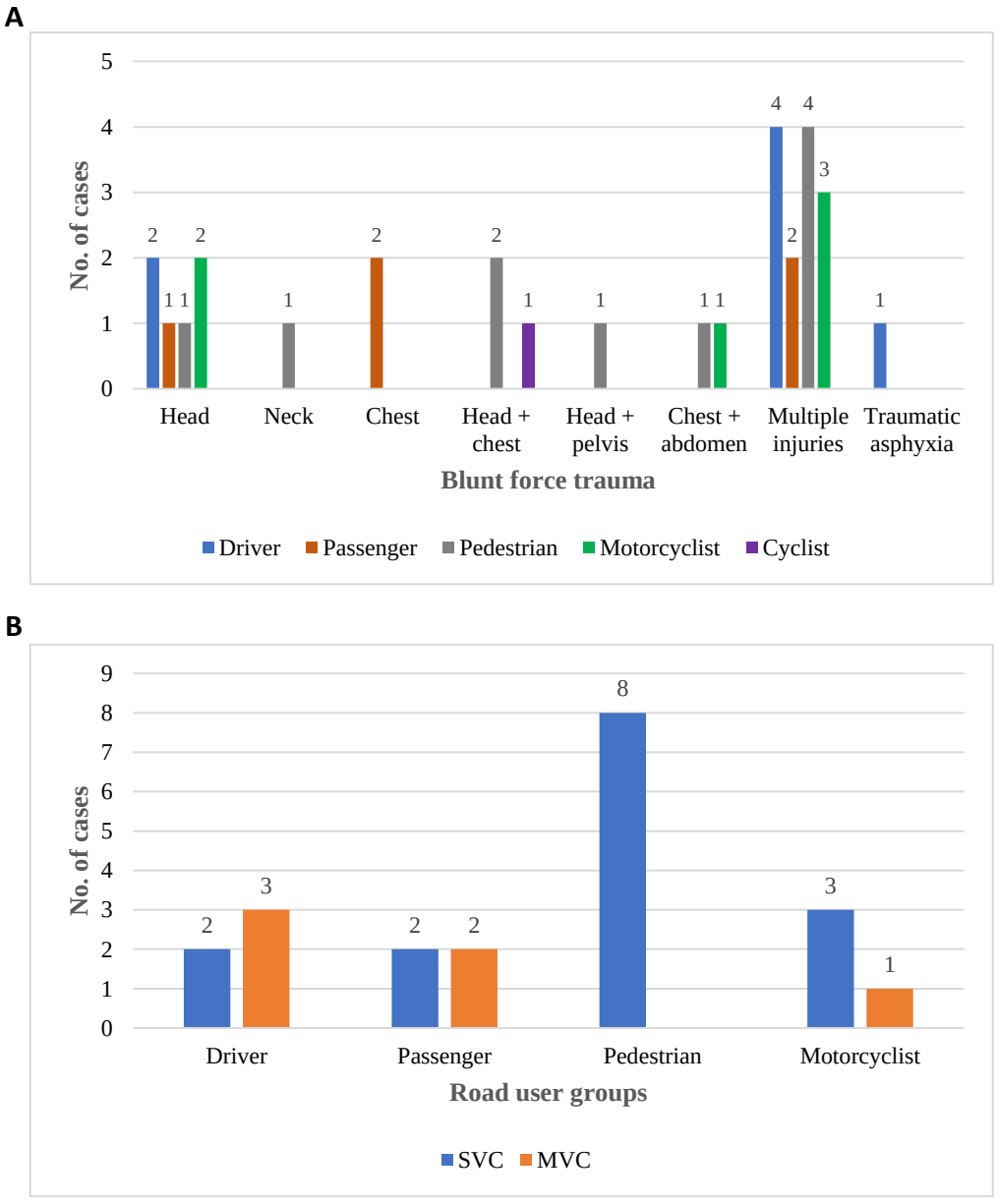


Figure 5.3: Distribution of (A) cause of death by blunt force trauma to various body parts and (B) SVC and MVC in the road user groups.

Chapter 6: Discussion and Conclusions

RTFs are a common occurrence across the world and contribute widely to the burden of injury and mortality. There is an increased risk of being involved in an RTA as a road user, when impaired by drugs and/or alcohol. This may result in death, in which the presence of drugs and alcohol can be detected and interpreted by forensic toxicologists.

In the South African forensic setting, there is no standardised protocol for toxicological analysis in RTA fatalities, despite publication of international recommendations of toxicological analysis in road user deaths. While blood for alcohol is largely routinely collected in RTA deaths in the WC, in SA, the potential involvement of substances (drugs other than alcohol) in the contribution or COD in road traffic victims cannot be overlooked.

The systematic literature review (Chapter 2) on the global reported prevalence of drugs of abuse in RTFs revealed that most research on this topic was conducted in developed countries, despite the largest burden of RTFs occurs in under-developed and developing nations. Additionally, the review highlighted the common occurrence of intoxicating drugs other than alcohol in victims of RTAs and the importance of forensic toxicological testing in these cases. Furthermore, the review highlighted the need for similar studies in such developing countries in which the findings could be compared both nationally and internationally. This pilot study was conducted to initiate the bridging of the gap in this area in SA.

Drug abuse is a problem in Cape Town, with research suggestion a wide prevalence of methamphetamine, cannabis and methaqualone (mandrax) use in the WC (in addition to alcohol). The presence of these, and other licit psychoactive drugs in the RTA population, is unknown within the South African forensic context.

To the author's knowledge, this pilot study was the first, to report on the toxicological findings in fatal RTAs in Cape Town, SA, and is in congruence with other research in the presence of substances (legal or illicit) in fatal RTAs.

6.1 A Cohort of Road Traffic Deaths from Salt River Mortuary

The study cohort comprised of 30 cases of which most were male, pedestrians and had an average age of 40 years old. The overall RTA deaths in the study period (n=110) were also predominantly male (88%). This correlates with other studies in which the proportion of males was higher than that of females [60, 63, 70]. Research investigating an individual's sex and driving behaviour have found that males tend to take more risks while driving than females [136, 137]. In addition, males are more likely to violate traffic laws (drinking and driving and driving above speed limits) than their female counterparts, thus suggesting their involvement in more traffic accidents [138]. Another aspect is that generally, the rates of alcohol and drug use and abuse are higher in men than in women, however, use, abuse or dependence patterns differ by drug and age group [139, 140]. In terms of drugs, alcohol consumption and abuse is significantly higher in men than in women of age group 25-34 years [141], although there has been a decrease in this gender gap over time [142]. Instead, women engage more in the non-medical use of prescription type drugs i.e. opioids and tranquilisers [143] particularly women of the older age group of 65 years and older age group [141]. Men are more likely to use cannabis in individual aged 18-24 years, with no variation in those aged 25 years [141] and older. whereas, the use of stimulants is similar between both sexes [144] across all age groups [141].

RTAs mostly affect individuals who are young and those in the working class in their prime working years. This age group is generally defined as those aged between 15-64 years [145]. Most of the cases in this study were specifically within the age range of 31-40 years with an average age of 40 years and this similar to the age range found in a study of drugs and fatal traffic accidents in the Czech Republic where the average age was in that study was 42.2 years [63]. A study by Al-Balbissi [146] found that highest accident rates were in 36-50 years age group and suggested that this might be attributed to the overconfidence of this age group in their driving abilities.

While the study size is small, pedestrians were identified as a vulnerable group, both in the cohort and the overall admissions in the study period. This is consistent with reports that this road user group has been noted to be a vulnerable group, particularly in developing countries [5, 13]. In studies reporting toxicological findings in all road user groups, some illustrate that pedestrians were the group mostly affected and the most prevalent drugs found in this vulnerable group were alcohol, stimulants (e.g. amphetamine, methamphetamine), cannabis and benzodiazepines [53, 57, 63, 64].

The demographic variables of this cohort of 30 cases were compared to those of the overall RTA cases admitted into SRM within the 4-month period. This was done to determine how representative the study cohort was to the actual fatal RTA population within the 4-month sample collection period at the SRM mortuary. Apart from the proportion of pedestrians - which in the overall RTA cases was significantly larger ($p < 0.05$) - there were no statistically significant differences between the other groups, suggesting that the study cohort was similar demographically to the overall RTA population.

Although the study cohort was similar to the larger 4-month overall RTA population, this does not account for the over population of RTF's in West-Metropole of Cape Town. The researcher recognises the limitation that this cohort may not be truly representative of the annual RTF admissions to Salt River and may not represent the larger Cape Town population. This requires wider investigations.

6.2 Post-mortem Drug Findings in the RTF Cohort

In the cohort of 30 cases investigated, 27 were positive for licit and/or illicit drugs, excluding alcohol, which was not the focus of this investigation. The most prevalent legal substances detected were caffeine and nicotinamide. Caffeine is a CNS stimulant which suppresses adenosine- a mediator of sleep [147] thus activating alertness. CNS stimulants have been reported as one of the most prevalent drug groups in fatal RTAs, but the specific drugs reported were amphetamines, methamphetamines, cocaine and methylenedioxymethamphetamine (ecstasy) [56, 63, 64, 82, 84]. Although caffeine has not been reported as prevalent drug in other similar studies, a study examined the effects of caffeine vs placebo on driving performance in regular consumers of caffeine who were deprived of caffeine prior to being tested. Interestingly, the findings suggest that increased alertness and improved driving performance are exaggerated effects of caffeine withdrawal as opposed to the effect of caffeine actually being beneficial [148]. Contrary to this though, another study also looking at effects of caffeine on driving found that one cup of caffeinated coffee had a positive effect on driving performance [149].

Nicotine activates the same reward pathways as other drugs of abuse (e.g. amphetamines, cocaine) but to a lesser extent [150] and produces small improvements in cognitive performance and attention [151]. Cotinine, which accumulates in the body due to tobacco exposure [152] can be looked at, along with nicotine to get an indication of tobacco smokers – whom, if deprived of

tobacco, can experience impairment in their cognition [153]. This impairment, due to deprivation, could potentially affect driving abilities.

Amphetamines are stimulants which have long been used medically to treat attention deficit hyperactivity disorder (ADHD) and narcolepsy but they do have a high potential of being abused [154]. This potential of abuse leads to drug related RTFs. A systematic review on the RTI or death risk due to amphetamine-type stimulants reported a moderate relationship between the use of amphetamine type stimulants and risk of fatally injury in an RTA, based on the 9 studies included in that review [155]. Additionally, even though this drug is a stimulant, other studies have reported on the driving impairment effects of at high doses [156, 157].

Nicotinamide, also known niacinamide is a form of vitamin B3 which is an OTC drug available as cosmetic, hair and skin preparation and can also be found in certain foods such as meat, dairy products, green vegetables and even coffee [158]. Associated use of OTC drugs and alcohol (a CNS depressant) can intensify the impairment effects of prescription/OTC drugs even though the drug may not ordinarily cause impairment on its own and patient are advised to not drive unless aware of individual response to medication [66]. OTC drugs have been detected in fatal RTA studies [60, 71] but none have detected nicotinamide.

Antihistamines, another group of OTC/prescription drugs, were frequently detected particularly among the drivers in this study. They are inverse agonists of histamines. Histamines are chemical messengers in the body which regulate the proliferation and differentiation of cells, regulate sleep/wake cycles, and alertness and attentiveness. First generation antihistamines such as chlorpheniramine, diphenhydramine and doxylamine may cause sedation, drowsiness, tiredness and lack of attention and concentration. Second generation antihistamines, however, have reduced effects on the CNS [159].

Vester and Volkerts reported that both first and second-generation antihistamines may significantly reduce driving performance after single and repeated consumption (without alcohol) [160]. Another study concurred with findings that both first and second-generation antihistamines may cause driving impairment, however, their conclusion was there was no significant relative risk associated with antihistamine use and traffic accidents [161]. As with other OTC/prescription drugs, the sedative effects of antihistamines is increased when used in combination with alcohol [162].

The two common illicit drugs detected were methaqualone and methamphetamine (and amphetamine its primary metabolite). Methaqualone (usually admixed with drugs such as diphenhydramine and orphenadrine) known as mandrax in SA is a sedative hypnotic drug which was used for insomnia, hypertension and anxiety due to its CNS depressant effects [163] This drug also has euphoric and aphrodisiac properties which contributed to its high recreational use and subsequent abuse [164]. In SA this drug is sold in tablet form and normally crushed, mixed with cannabis and smoked in a cannabis pipe, this is known as white pipe or 'wit pyp' [163, 165] The combination of the two drugs produces a greater "rush" effect [166]. Methamphetamine is another commonly abused synthetic drug in SA colloquially known as 'tik'. Tik is a highly addictive stimulant with euphoric and increased energy effects [167].

These drugs have been reported in other RTF cases. Two studies reported the presence of methamphetamines in fatally injured drivers, more so in truck drivers who drove long distances [82, 168]. In another study by Logan, the driving behaviour which led to the accident of methamphetamine-positive drivers was said to be more consistent with methamphetamine withdrawal whose effects are as problematic as the intoxication [169].

Methaqualone has been noted to cause significant driving impairment [170] but in another study, the presence of methaqualone in four drivers was determined to have not contributed to the accidents and in one case in which alcohol was also detected, the level of intoxication with alcohol would have likely led to the accident with or without the presence of methaqualone [33].

Opioids/opiates (i.e morphine, hydrocodone and codeine), zopiclone and amitriptyline all have sedative effects in common, although they classify under different drug groups. Opioid use was found to significantly increase crash involvement and culpability [171] and codeine having a dose dependent effect which may lead to driving impairment [172]. The acute doses of amitriptyline significantly reduced driver vigilance and impaired driving performance [173]. Similarly, zopiclone also reduced driver performance, however, less so for chronic users compared to new users [174, 175].

It is important to note that the presence of drugs detected does not necessarily correlate to the level of intoxication of the user at the time of death, nor was it an indicator (particularly of drugs known to have impairing effects), that the drug was the causal factor of the accident. Quantification of the drugs and further research into effects of drugs as well as an evaluation of the case histories and circumstantial evidence of the accidents would be required to be able to make conclusions pertaining to the possible involvement of the drugs detected in the fatal RTAs of this study.

6.2.1 Case Study

While the exact role of drugs cannot be ascertained, this study provides a proof of concept of the importance of drug testing in RTFs. This can be illustrated by the following specific cases derived from the cohort.

Case 8:

The deceased was a 19-year-old male driver. The deceased was the only victim involved in an MVC on a wet, tar road with speed limit of 120 km/h. Toxicology analysis revealed chlorpheniramine, diphenhydramine and codeine detected in the blood. Blood alcohol analysis (performed by the external FCL laboratory) was negative.

Substances detected in blood were antihistamines and the opiate, codeine. These drugs can have possible impairing effects such as causing sedation. The drug testing performed in this study revealed the presence of these impairing drugs in this case which otherwise would not have been detected as samples for these cases (RTA cases) had only been sent for BAC analysis, as is routinely done. The nature of the role of these drugs in contributing the death would require further investigation, including the quantitation of those substances together with further evaluation by the forensic toxicologist and pathologist.

6.3 Road user groups, medicolegal findings and collision types

The two most observed CODs overall were head trauma and multiple trauma injuries to the body. Similar to the findings in this study, other studies found that one of the most common causes of death was injuries to the head [176-178]. Passengers in this study mostly succumbed to blunt force trauma to the chest. A proposed reason for this is that the lack of a steering wheel may result in a much greater forward momentum and the impact and force is directed right on the chest and abdomen of the passenger [179].

The SVCs were more prevalent than the MVCs. These SVCs happened on tar roads, in good condition with speed limits ranging from 60-120 km/h. In terms of motorised vehicles, these collisions were mostly motorcyclists (10%) who had caffeine, nicotine and cotinine (stimulants) and zopiclone (sedative hypnotic) detected. One motorcyclist had no drugs detected. The other road user group involved in SVCs were pedestrians (50%) with same drug groups detected. This might suggest a positive relationship between impairment by road users and drugs [56].

6.4 Limitations

As with all research, there were some limitations in this study. Although this was a pilot study, the sample size of 30 cases constrained the investigation into demographic distribution and toxicological and medicolegal findings of the cases. These cases may not represent the overall RTF cohort admitted to SRM annually, nor the WC mortuary population. In addition, while insight into the drugs detected is provided, this study did not provide a true representation of the drugs that may or may not be within the WC population of road users.

Obtaining consent from the next-of-kin, which is essential in forensic research and investigating such vulnerable populations, was a challenge in this project. This process required a collaborative effort with the mortuary Forensic Pathology Officers (FPOs), who would notify the researcher when a family for an eligible case was at the mortuary. However, due to the FPOs large workload, a substantial number of families were missed, and consent was unable to be obtained. Along with this, there was a time constraint in that the sample collection period was limited to 4 months based on the timeline of the research project as a whole. Getting consent is important to conduct research where the outcomes are currently unknown and improving this process of notification would be of value to future projects. One suggestion to overcome this is for the researcher to screen the files of the cases admitted to the mortuary daily and mark the eligible cases which would serve as a reminder for the FOs to contact the researcher should the next-of-kin arrive at the mortuary and secondary to that, for the researcher to be "based" at the mortuary premises/facility throughout the specimen collection period. In addition, a longer specimen collection period could increase the number of cases obtained for such a study.

While the LC-QTOF/MS provides screening capabilities in high resolution, a quantitative confirmation would be required in these cases, especially in routine forensic practice. This would provide improved probative weight to the findings and allow for greater interpretation by the forensic toxicologist. The research is also cognisant of the importance of validation of analytical methods. This study did not permit for validation procedures to be carried out, and the method that is currently available at the Division of Pharmacology was used as developed. This limitation is one that would need to be accounted for in routine service and especially in quantitative methods, where validation for the specific drugs in the post-mortem specimens would be required.

The limit of detection for concentrations of THC from cannabis use was high on the LC-QTOF/MS and it was recommended that urine be analysed on an immunoassay. This would only illustrate

exposure, however, and won't provide an indication of timing of use nor provide any indication of impairment. In future the method needs to be validated for THC, 11-nor-9-carboxy-delta 9-tetrahydrocannabinol (THCCOOH) and 11-hydroxy tetrahydrocannabinol (THCOH) to determine the prevalence and concentrations of THC in these cases.

Because of the small study size and the descriptive nature of this study, performing certain statistical tests to determine significant differences or lack thereof of prevalence of drugs between the different road user groups was not possible. Such statistical tests are important in adding strength and certainty to the findings and the conclusions thereof and could be undertaken with a more comprehensive sample size.

Another limitation to the study was the lack of a control group. All the specimens tested were from a deceased RTA cohort. An ideal control group would be living drivers who were pulled over randomly for drug testing and/or for DUI. Such a control would allow for comparison of drug prevalence between the deceased RTA population and the general road user population. Lack of such a control group means it is also not possible to infer whether the prevalence of drug use is higher among the deceased road users or the general road user population.

6.5 Future research

The preliminary findings in this study have highlighted the need for future research in this area. The first of which would be to continue this research in a larger study cohort and investigate the drug trends in these cases.

The BACs were not discussed in this project, as these were performed by the National Health FCL. Including these results when available to include the relevance of both drugs and/or alcohol in these cases is essential in going forward to recommend best practice. This will assist in understanding of the extent of the relationship between alcohol and drugs and their additive effects in causing impairment in the different road users within the local context. This pilot study provided important information concerning the requirements for developing future research and the roll-over into best practice within the WC.

6.6 Conclusion

This study was a pilot study to investigate the toxicological findings in a cohort of RTA fatalities in the West Metropole of Cape Town and gain insight into the drugs detected in such cases. Although the findings are limited, this study has brought to the forefront that drugs are present in the road user population and further insight into the role of these drugs together with alcohol in RTFs is required. Most cases were positive for a substance (other than alcohol – which was not investigated in this study), and prescription drugs appeared to dominate the cohort. Many drugs with psychoactive and/or sedative/hypnotic properties were identified within the cohort.

Despite the fact that the study size was small, and no inferences can be made about the general population, the results of this study have set a basis for future research which would most certainly contribute to the body of literature on this topic - considering that such a study had not yet been done in the South African setting.

It is important to reiterate that with nature of this study, the presence of the drugs at the time of death do not infer on causation of the accidents. After all, RTAs are multifactorial and to determine the role that drugs might have had in the accident is complex and would require more rigorous investigations and interpretive expertise. A key aspect to keep in mind in future research is using the information to develop a standardised protocol for the routine collection and analyses of specimens for such cases, specifically suited for the context and setting of SA.

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Appendices

Appendix A - Scopus search strategy

Search #	Search texts and syntaxes
#1	TITLE-ABS-KEY (road user OR road users OR driver OR drivers OR passenger OR passengers OR motorcyclist OR motorcyclists OR cyclist OR cyclists OR pedestrian OR pedestrians)
#2	TITLE-ABS-KEY (drug OR drugs OR illicit drug OR illicit drugs OR licit drug OR licit drugs)
#3	TITLE-ABS-KEY (illegal drug OR illegal drugs OR legal drug OR legal drugs)
#4	TITLE-ABS-KEY (substance OR substances OR non-alcoholic drugs OR non alcoholic drugs)
#5	TITLE-ABS-KEY (street drug OR streets drugs OR recreational drug OR recreational drugs)
#6	TITLE-ABS-KEY (psychotropic drug OR psychotropic drugs OR drug of abuse OR drugs of abuse)
#7	TITLE-ABS-KEY (psychoactive drug OR psychoactive drugs OR psychotic drug OR psychotic drugs)
#8	#2 OR #3 OR #4 OR #5 OR #6 OR #7
#9	TITLE-ABS-KEY (road traffic accident OR road traffic accidents OR traffic accident OR traffic accidents)
#10	TITLE-ABS-KEY (motor vehicle accident OR motor vehicle accidents OR motor vehicle crash OR motor vehicle crashes OR motor-vehicle crash OR motor-vehicle crashes)
#11	TITLE-ABS-KEY (road-traffic crash OR road-traffic crashes OR road traffic crash OR road traffic crashes OR traffic crash OR road crash OR road crashes)
#12	TITLE-ABS-KEY (traffic collision OR traffic collisions OR road traffic collision OR road traffic collisions)
#13	#9 OR #10 OR #11 OR #12
#14	TITLE-ABS-KEY (fatality OR mortality OR casualty OR death OR fatal OR dead OR die OR died)
#15	#1 AND #8 AND #13 AND #14

Appendix B – Final search terms and filters for all databases

Database	Search terms	Articles retrieved	Articles included
PubMed	Sort by: Most recent (((((fatality OR mortality OR casualty OR death OR fatal OR dead OR die OR died))) AND ((road traffic accident OR road traffic accidents OR traffic accident OR traffic accidents OR motor vehicle accident OR motor vehicle accidents OR motor vehicle crash OR motor vehicle crashes OR motor-vehicle crash OR motor-vehicle crashes OR road-traffic crash OR road-traffic crashes OR road traffic crash OR road traffic crashes OR traffic crash OR road crash OR road crashes OR traffic collision OR traffic collisions OR road traffic collision OR road traffic collisions))) AND ((((drug OR drugs OR illicit drug OR illicit drugs OR licit drug OR licit drugs OR illegal drug OR illegal drugs OR legal drug OR legal drugs OR substance OR substances OR non-alcoholic drugs OR non alcoholic drugs OR recreational drug OR recreational drugs OR drug of abuse OR drugs of abuse OR psychoactive drug OR psychoactive drugs OR psychotic drug OR psychotic drugs OR street drug OR psychotropic drug)) OR "Psychotropic Drugs"[MeSH]) OR "Street Drugs"[MeSH])) AND (((((((road user OR road users)) OR (driver OR drivers)) OR (passenger OR passengers)) OR (cyclist OR cyclists)) OR (pedestrian OR pedestrians)) OR (motorcyclist OR motorcyclists	380	35
Scopus	(TITLE-ABS-KEY (road AND user OR road AND users OR driver OR drivers OR passenger OR passengers OR motorcyclist OR motorcyclists OR cyclist OR cyclists OR pedestrian OR pedestrians)) AND ((TITLE-ABS-KEY (drug OR drugs OR illicit AND drug OR illicit AND drugs OR licit AND drug OR licit AND drugs)) OR (TITLE-ABS-KEY (illegal AND drug OR illegal AND drugs OR legal AND drug OR legal AND drugs)) OR (TITLE-ABS-KEY (substance OR substances OR non-alcoholic AND drugs OR non AND alcoholic AND drugs)) OR (TITLE-ABS-KEY (street AND drug OR streets AND drugs OR recreational AND drug OR recreational AND drugs)) OR (TITLE-ABS-KEY (psychotropic AND drug OR psychotropic AND drugs OR drug AND of AND abuse OR drugs AND of AND abuse)) OR (TITLE-ABS-KEY (psychoactive AND drug OR psychoactive AND drugs OR psychotic AND drug OR psychotic AND drugs))) AND (((TITLE-ABS-KEY (road-traffic AND crash OR road-traffic AND crashes OR road AND traffic AND crash OR road AND traffic AND crashes OR traffic AND crash OR road AND crash OR road AND crashes)) OR (TITLE-ABS-KEY (traffic AND collision OR traffic AND collisions OR road AND traffic AND collision OR road AND traffic AND collisions))) OR ((TITLE-ABS-KEY (road AND traffic AND accident OR road AND traffic AND accidents OR traffic AND accident OR traffic AND accidents)) OR (TITLE-ABS-KEY (motor AND vehicle AND accident OR motor AND vehicle AND accidents OR motor AND vehicle AND crash OR motor AND vehicle AND crashes OR motor-vehicle AND crash OR motor-vehicle AND crashes)))) AND (TITLE-ABS-KEY (fatality OR mortality OR casualty OR death OR fatal OR dead OR die OR died))	214	1
Web of Science	Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years TITLE: (road user OR road users OR driver OR drivers OR passenger OR passengers OR motorcyclist OR motorcyclists OR cyclist OR cyclists OR pedestrian OR pedestrians) AND TITLE: (drug OR drugs OR illicit drug OR illicit drugs OR licit drug OR licit drugs OR illegal drug OR illegal drugs OR legal drug OR legal drugs OR substance OR substances OR non-alcoholic drugs OR non alcoholic drugs OR street drug OR streets drugs OR recreational drug OR recreational drugs OR psychotropic drug OR psychotropic drugs OR drug of abuse OR drugs of abuse OR psychoactive drug OR psychoactive drugs OR psychotic drug OR psychotic drugs) AND TITLE: (road traffic accident OR road traffic accidents OR traffic accident OR traffic accidents OR motor vehicle accident OR motor vehicle accidents OR motor vehicle crash OR motor vehicle crashes OR motor-vehicle crash OR motor-vehicle crashes OR road-traffic crash OR road-traffic crashes OR road traffic crash OR road traffic crashes OR traffic crash OR road crash OR road crashes OR traffic collision OR traffic collisions OR road traffic collision OR road traffic collisions) AND TITLE: (fatality OR mortality OR casualty OR death OR fatal OR dead OR die OR died)	7	1
CINAHL	Limiters - Full Text; Peer Reviewed Search modes - Boolean/Phrase (road user OR road users OR driver OR drivers OR passenger OR passengers OR motorcyclist OR motorcyclists OR cyclist OR cyclists OR pedestrian OR pedestrians) AND (drug OR drugs OR illicit drug OR illicit drugs OR licit drug OR licit drugs OR illegal drug OR illegal drugs OR legal drug OR legal drugs OR substance OR substances OR non-alcoholic drugs OR non alcoholic drugs OR street drug OR streets drugs OR recreational drug OR recreational drugs OR psychotropic drug OR psychotropic drugs OR drug of abuse OR drugs of abuse OR psychoactive drug OR psychoactive drugs OR psychotic drug OR psychotic drugs) AND (road traffic accident OR road traffic accidents OR traffic accident OR traffic accidents OR motor vehicle accident OR motor vehicle accidents OR motor vehicle crash OR motor vehicle crashes OR motor-vehicle crash OR motor-vehicle crashes OR road-traffic crash OR road-traffic crashes OR road traffic crash OR road traffic crashes OR traffic crash OR road crash OR road crashes OR traffic collision OR traffic collisions OR road traffic collision OR road traffic collisions) AND (fatality OR mortality OR casualty OR death OR fatal OR dead OR die OR died)	5	0
Grey literature	Search : keywords No limits set (road user OR road users OR driver OR drivers OR passenger OR passengers OR motorcyclist OR motorcyclists OR cyclist OR cyclists OR pedestrian OR pedestrians) AND (drug OR drugs OR illicit drug OR illicit drugs OR licit drug OR licit drugs OR illegal drug OR illegal drugs OR legal drug OR legal drugs OR substance OR substances OR non-alcoholic drugs OR non alcoholic drugs OR street drug OR streets drugs OR recreational drug OR recreational drugs OR psychotropic drug OR psychotropic drugs OR drug of abuse OR drugs of abuse OR psychoactive drug OR psychoactive drugs OR psychotic drug OR psychotic drugs) AND (road traffic accident OR road traffic accidents OR traffic accident OR traffic accidents OR motor vehicle accident OR motor vehicle accidents OR motor vehicle crash OR motor vehicle crashes OR motor-vehicle crash OR motor-vehicle crashes OR road-traffic crash OR road-traffic crashes OR road traffic crash OR road traffic crashes OR traffic crash OR road crash OR road crashes OR traffic collision OR traffic collisions OR road traffic collision OR road traffic collisions) AND (fatality OR mortality OR casualty OR death OR fatal OR dead OR die OR died)	33	0

Appendix C – Adapted JBI Extraction Form

Study ID
Author
Article title
Year published
Country
Study period
Aims
Study design
Population of interest
Sample size studied

Methods (toxicological analyses and statistical tests)
Tests done in methods
Instruments used
Statistical significance (p-value or CI)
Measurement of outcome variables (prevalence or prevalence odds ratio/rates)
Outcome variables

Results
Basic demographics data
Prevalence n/N (%)
Incidence n/N (%)
Specific drugs

Exclusions in population of interest
Study limitations
Ethical approval attained
Informed consent
Appraisal quality rating

Appendix D – AXIS Critical Appraisal Tool for Cross-sectional Studies

Section	Question	Yes	No	Don't know/ Comment
Introduction				
1	Were the aims/objectives of the study clear?			
Methods				
2	Was the study design appropriate for the stated aim(s)?			
3	Was the sample size justified?			
4	Was the target/reference population clearly defined? (Is it clear who the research was about?)			
5	Was the sample frame taken from an appropriate population base so that it closely represented the target/reference population under investigation?			
6	Was the selection process likely to select subjects/participants that were representative of the target/reference population under investigation?			
7	Were measures undertaken to address and categorise non-responders?			
8	Were the risk factor and outcome variables measured appropriate to the aims of the study?			
9	Were the risk factor and outcome variables measured correctly using instruments/measurements that had been trialled, piloted or published previously?			
10	Is it clear what was used to determined statistical significance and/or precision estimates? (e.g. p-values, confidence intervals)			
11	Were the methods (including statistical methods) sufficiently described to enable them to be repeated?			
Results				
12	Were the basic data adequately described?			
13	Does the response rate raise concerns about non-response bias?			
14	If appropriate, was information about non-responders described?			
15	Were the results internally consistent?			
16	Were the results presented for all the analyses described in the methods?			
Discussion				
17	Were the authors' discussions and conclusions justified by the results?			
18	Were the limitations of the study discussed?			
Other				
19	Were there any funding sources or conflicts of interest that may affect the authors' interpretation of the results?			
20	Was ethical approval or consent of participants attained			

Quality rating (Good, fair or poor)	
Rater's initials	
Additional comments (if poor please state why)	

Appendix E – List of all articles included in the review

Reference number	First author	Article title	Year published	Country
[40]	Bogstrand *et al*	Associations between driving under the influence of alcohol or drugs, speeding and seatbelt use among fatally injured car drivers in Norway.	2015	Norway
[41]	Brady *et al.*	Prevalence of alcohol and other drugs in fatally injured drivers.	2013	US
[42]	Carmen del Rio *et al.*	Alcohol, illicit drugs and medicinal drugs in fatally injured drivers in Spain between 1991 and 2000.	2002	Spain
[43]	Christophersen *et al*	Prevalence of alcohol and drugs among motorcycle riders killed in road crashes in Norway during 2001-2010.	2015	Norway
[44]	Drummer *et al.*	The incidence of drugs in drivers killed in Australian road traffic crashes.	2003	Australia
[45]	Isalberti *et al.*	Prevalence of alcohol and other psychoactive substances in injured and killed drivers	2011	Finland, Norway, Portugal, Sweden
[46]	Legrand *et al.*	Prevalence of alcohol, illicit drugs and psychoactive medicines in killed drivers in four European countries.	2014	Finland, Norway, Portugal, Sweden
[47]	Poulsen *et al.*	The incidence of alcohol and other drugs in drivers killed in New Zealand road crashes 2004-2009.	2012	New Zealand
[48]	Rudisill *et al.*	Trends in drug use among drivers killed in U.S. traffic crashes, 1999-2010.	2014	US
[49]	Rudisill *et al*	Characterization of drug and alcohol use among senior drivers fatally injured in U.S. motor vehicle collisions, 2008-2012.	2016	US
[50]	Schwilke *et al.*	Changing patterns of drug and alcohol use in fatally injured drivers in Washington State.	2006	US
[51]	Ahlm *et al.*	Alcohol and drugs in fatally and non-fatally injured motor vehicle drivers in northern Sweden.	2009	Sweden
[52]	Ahlner *et al.*	Prevalence of alcohol and other drugs and the concentrations in blood of drivers killed in road traffic crashes in Sweden.	2014	Sweden
[53]	Al-Abdallat *et al.*	The prevalence of alcohol and psychotropic drugs in fatalities of road-traffic accidents in Jordan during 2008-2014.	2016	Jordan
[54]	Battah *et al.*	Alcohol and psychoactive drugs in road traffic fatalities within northern district of Amman	2013	Jordan
[56]	Cheng *et al.*	An epidemiological study on alcohol/drugs related fatal traffic crash cases of deceased drivers in Hong Kong between 1996 and 2000.	2004	Hong Kong
[57]	Elliott *et al.*	The prevalence of drugs and alcohol found in road traffic fatalities: a comparative study of victims.	2009	UK
[58]	Gjerde *et al.*	Incidence of alcohol and drugs in fatally injured car drivers in Norway.	1993	Norway
[59]	Gjerde *et al.*	Toxicological investigations of drivers killed in road traffic accidents in Norway during 2006-2008.	2011	Norway
[60]	Hamnett *et al.*	Toxicological findings in driver and motorcyclist fatalities in Scotland 2012-2015.	2017	Scotland
[61]	Jones *et al.*	Five-year update on the occurrence of alcohol and other drugs in blood samples from drivers killed in road-traffic crashes in Sweden.	2009	Sweden
[62]	Mercer *et al.*	Alcohol, drugs, and impairment in fatal traffic accidents in British Columbia.	1995	Canada
[63]	Mravčík *et al.*	Drugs and fatal traffic accidents in the Czech Republic.	2007	Czech Republic
[64]	Pelição *et al*	Predominance of alcohol and illicit drugs among traffic accidents fatalities in an urban area of Brazil.	2016	Brazil
[65]	Terhune *et al.*	The Incidence and Role of Drugs in Fatally Injured Drivers	1992	US

[66]	Woodall *et al*	Toxicological Findings in Fatal Motor Vehicle Collisions in Ontario, Canada: A One-Year Study	2015	Canada
[67]	Afzali *et al.*	Frequency of alcohol and substance abuse observed in drivers killed in traffic accidents in Hamadan, Iran.	2013	Iran
[68]	Caplan *et al.*	Drugs in Driver Fatalities: A preliminary study in the state of Maryland	1990	US
[69]	Cimbura *et al.*	Incidence and Toxicological Aspects of Drugs Detected in 484 Fatally Injured Drivers and Pedestrians in Ontario	1982	Canada
[70]	Costa *et al.*	Prevalence of ethanol and illicit drugs in road traffic accidents in the centre of Portugal: An eighteen-year update.	2012	Portugal
[71]	Crouch *et al.*	The prevalence of drugs and alcohol in fatally injured truck drivers.	1993	US
[72]	Fortenberry *et al.*	Analysis of drug involvement in traffic fatalities in Alabama	1986	US
[73]	Garriott *et al.*	Incidence of drugs and alcohol in fatally injured motor vehicle drivers.	1977	US
[74]	Holmgren *et al.*	Alcohol and drugs in drivers fatally injured in traffic accidents in Sweden during the years 2000-2002.	2004	Sweden
[75]	Logan *et al.*	Drug and alcohol use in fatally injured drivers in Washington State.	1996	US
[77]	Sidlo J.	Psychoactive substance-related deaths in road traffic accidents in Slovakia between 2000 and 2007.	2009	Slovakia
[78]	Turnbridge *et al.*	The incidence of drugs in road accident fatalities in Great Britain	1990	UK
[79]	Warren *et al.*	Drugs detected in fatally injured drivers in the province of Ontario	1981	Canada
[80]	Williams *et al.*	Drugs in fatally injured young male drivers.	1985	US
[81]	Woodhouse *et al.*	The Incidence of Drugs in Fatally Injured Drivers	1974	US
[83]	Seymour *et al.*	Role of drugs and alcohol in impaired drivers and fatally injured drivers in the Strathclyde police region of Scotland, 1995-1998.	1999	UK
[84]	Carmen del Rio *et al.*	Presence of illegal drugs in drivers involved in fatal road traffic accidents in Spain.	2000	Spain

Appendix F – Human Research Ethics Approval Letter

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

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone (021) 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

22 May 2018

HREC REF: 270/2018

Ms B Davies
Division of Forensic Medicine & Toxicology
Entrance 3, Level 1
Falmouth Building-FHS

Dear Ms Davies

PROJECT TITLE: TOXICOLOGICAL FINDINGS IN FATAL TRANSPORT ACCIDENT IN CAPE TOWN: PILOT STUDY- LINKED TO 324/2015 (MPhil candidate- Nondumiso Shongwe)

Thank you for your response letter dated 14 May 2018, addressing the issues raised by the Human Research Ethics Committee (HREC).

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

Approval is granted for one year until the 30 May 2019.

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

We acknowledge that the student: Nondumiso Shongwe will also be involved in this study.

Please quote the HREC REF in all your correspondence.

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

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

Signature Removed

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

HREC 270/2018

Appendix G –Information Sheet for Participant Next-of-kin

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INFORMATION SHEET FOR PARTICIPANTS' NEXT-OF-KIN

Research Project:

Toxicological Findings in Fatal Road Traffic Accidents in Cape Town: A Pilot Study

Supervisor: B. Davies | **Co-supervisor**: K. Auckloo | **Researcher:** N. Shongwe
Division of Forensic Medicine and Toxicology, University of Cape Town

Who are we?

I, Nondumiso Shongwe, am an MPhil Biomedical Forensic Science candidate doing a research study with my supervisors on Road Traffic Accidents in Cape Town, South Africa. This information sheet will be used to interview and obtain consent from the family/relative/next-of-kin of the decedent involved in a road traffic accident and then admitted to the Salt River mortuary.

What is the purpose of the study?

Road Traffic Accidents (RTAs) are very common in South Africa and, unfortunately, often lead to the death of the individuals involved. One of the major contributors to fatal road accidents is the influence of alcohol and drugs (legal or illegal) on road users (e.g. drivers, passengers, pedestrians, motorcyclists and cyclists).

There are limited to no information on substance use in RTAs in our country. However, the available information (especially from treatment centres) report that alcohol is the most commonly used substance followed by illegal drugs such as dagga, tik, heroin and cocaine.

What is the study about?

In this study, we will be testing for substances other than alcohol in biological samples (blood, urine, eye fluid and hair) collected during the routine autopsy of decedents due to fatal road accidents, which is a postmortem examination of all individuals who have passed away and are admitted to the Salt River mortuary.

In a normal situation, when an individual is admitted to the Salt River mortuary following a death incident, the decedent's family, relative or next-of-kin is called to identify the body of the individual. The informed consent interview typically occurs just before or after the body identification. Then, the collection of samples takes place during the routine scheduled postmortem examination of the decedent. The decedents

name and personal details will be removed from the information and samples collected for the study and no one will be able to identify them.

After collection, the samples will be sent to the testing laboratory at the University of Cape Town Division of Clinical Pharmacology in Groote Schuur Hospital. The samples will be analyzed for substances and the results produced will remain confidential and will not be shared with anyone. These results will be stored in an access controlled database at University of Cape Town.

Why are you being interviewed?

You have been asked to be interviewed because you are presenting to the Salt River mortuary after your family member / next-of- kin / relative has passed away in a RTA, which is the topic of our study. We will be looking at which substances are more commonly found in road traffic fatalities within our communities and understand their role in road accidents. Please remember that the results are confidential and will not alter any criminal procedures or outcomes. Our findings will help in the intervention, management and prevention of road traffic accidents in Cape Town.

Do you have to agree to the collection of samples?

You are invited to give permission for the collection of samples from your family member, relative or next-of-kin. Your contribution is completely voluntary and at no cost to you at all. You are free to not take part. Should you be willing to give consent, you will be asked to indicate this by signing a consent form. You may withdraw your consent from the study at any time, for any reason. Your decision on whether or not to give consent will not affect any services provided to you by the Forensic Pathology Services.

What if you come to the mortuary much later?

As mentioned earlier, the autopsy and collection of samples usually happen after obtaining consent and identifying the body. However, sometimes the scheduled autopsy procedure is performed before body identification and thus, the interview for obtaining consent is done after the samples have already been collected. In such cases, the samples will be collected and stored until such a time the **delayed consent** is obtained from the decedent's next-of-kin / relative / family. In situations where obtaining **delayed consent** is unsuccessful, the respective samples will be destroyed. This will not affect the results of the autopsy procedure. Once again, your help is completely voluntary and you are free to refuse.

What happens to the results?

The results of this study will only be available to the researchers and the doctor who performed the autopsy. Specific results will not be provided to the family, relative or next-of-kin. At completion, the overall

findings may be published in a research journal and/or presented at meetings/conference, however, confidential information will not be shared in any capacity and no one will be able to link the results to the decedents.

How will samples be stored and used in future studies?

During the study period, biological samples will be stored according to the UCT Division of Medicine and Forensic Toxicology's standard operating procedures for biological sample storage.

Future research can be understood as one or more studies of possible future events and/or circumstances. The findings of this study may create new questions or ideas that should be investigated to contribute to the knowledge gap in forensic toxicology. For example, looking at a specific drug, specimen comparisons, and different instrumental analyses, among others.

Given that this study forms part of the basis for future research in post mortem forensic toxicology in South Africa, samples collected from decedents in which consent is obtained for 'storage of samples for future research' will be transferred after the study period and stored in the Forensic Toxicology Unit laboratory at the University of Cape Town according to their standard sample long-term storage protocol. Where consent was not obtained, the samples will be destroyed after completion of the study.

Ethical Approval?

The research study was reviewed and approved by the University of Cape Town, Faculty of Health Sciences, Human Research Ethics Committee. This committee is responsible for protecting the rights of individuals who volunteer for participation in research studies.

If you agree to take part, and have any further queries about the study please contact:

Supervisor: Bronwen Davies | **Tel:** 021 406 6026 | **Email:** bronwen.davies@uct.ac.za

Co-supervisor: Kathrina Auckloo | **Cell:** 072 642 1245 | **Email:** ackmar005@myuct.ac.za

Researcher: Nondumiso Shongwe |**Cell:** 073 709 2663 | **Email:** SHNNON012@myuct.ac.za

Human Research Ethics Committee | ***Tel:*** *021 406 6492* | ***Email:*** *sumaya.ariefdien@uct.ac.za*

If you agree to participate in this study, please read and sign the consent form attached.

Appendix H – Consent Form

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CONSENT FORM

I, _____ (full name of next-of-kin), the spouse/partner/major child/parent/guardian/major brother/major sister (circle relationship) of the deceased with case number WC11/_____/_____,

I confirm that:	Yes	No
1. I have read and understood the information provided on the information sheet	☐	☐
2. I understand that participation is voluntary	☐	☐
3. I am aware that I may withdraw from the study at any time without reason or consequence whether before or during the study	☐	☐
4. Anonymity will be maintained and neither the deceased or my family will be identified	☐	☐

I consent to:		
1. The collection of biological samples and their toxicological analyses for the presence and quantification of drugs	☐	☐
2. Samples being stored for future use and	☐	☐
3. Samples being used for future research with ethical approval from appropriate ethics committee	☐	☐

_____ _____

Signature of next of kin Date

_____

Full Name of person obtaining consent

_____ _____

Signature of person obtaining consent Date

Appendix I – Toxicological analysis results for 30 case study cohort

Case number	Age	Sex	Road User	Cause of Death	Samples collected	Samples in which drugs were detected	Results
001	37	Male	Passenger	Multiple blunt force injuries	Blood, urine, VH	None	Negative
002	39	Male	Pedestrian	Blunt force neck trauma	Blood, urine, VH	All collected samples	9-Hydroxyrisperidone, Chinine, Methaqualone, Quinine
003	45	Male	Pedestrian	Blunt force injuries to head and chest	Blood, VH	All collected samples	Methaqualone
004	51	Male	Motorcyclist	Multiple blunt force trauma	Bile, VH	All collected samples	Acetaminophen, Ketamine, Lidocaine, Norketamine, Tranexamic acid, Warfarin, Chlorpheniramine, Morphine, Hydromorphone
005	40	Male	Cyclist	Blunt force injuries to head and chest	Blood, VH, urine	Urine	Hydromorphone, Morphine
006	52	Male	Driver	Multiple blunt force injuries	Blood, VH, urine	All collected samples	Diphenhydramine, Orphenadrine
007	54	Male	Passenger	Blunt force chest trauma	Blood, bile, VH	None	Negative
008	19	Male	Driver	Multiple blunt force injuries	Blood, VH, urine	All collected samples	Chlorpheniramine, Codeine, Diphenhydramine
009	25	Male	Motorcyclist	Blunt force head injury	Blood, urine, VH	None	Negative
010	45	Male	Pedestrian	Blunt force head and chest injury	Blood, urine, VH	All collected samples	Amitriptyline, Caffeine, Nicotinamide, Cotinine and Nicotine
011	30	Male	Pedestrian	Multiple blunt force injuries	Blood, bile, VH	All collected samples	Caffeine, Nicotinamide
012	45	Male	Passenger	Blunt force chest trauma	Blood	Blood	Caffeine, Cotinine
013	29	Male	Pedestrian	Blunt force head trauma	Blood	Blood	Caffeine, Cotinine, Nicotinamide
014	43	Female	Driver	Multiple blunt force injuries	Blood, VH	All collected samples	Caffeine, Chlorpheniramine
015	26	Male	Driver	Head injury	Blood, VH, urine	All collected samples	Acetaminophen, Caffeine, Cotinine, Nicotinamide, Nicotine
016	34	Male	Motorcyclist	Multiple blunt force injuries	Blood, VH, urine	All collected samples	Caffeine, Gliclazide, Nicotinamide
017	33	Male	Motorcyclist	Blunt force head injury	Blood, VH, bile	All collected samples	Caffeine, Nicotinamide
018	35	Male	Pedestrian	Multiple blunt force injuries	Blood, bile	All collected samples	Amphetamine, Caffeine, Nicotinamide, Caffeine, Cotinine, Diphenhydramine, Methamphetamine, Methaqualone, Nicotine
019	43	Female	Passenger	Blunt force head trauma	Blood	Blood	Acetaminophen, Caffeine, Chlorpheniramine, Nicotinamide
020	62	Female	Passenger	Multiple blunt force injuries	Blood	Blood	Acetominophen, Caffeine, Lidocaine
021	36	Male	Pedestrian	Multiple blunt force injuries	Blood, VH, urine	All collected samples	Caffeine, Cotinine, Nicotinamide, Nicotine
022	26	Male	Motorcyclist	Blunt force injuries to chest and abdomen	Blood, VH, bile	All collected samples	Caffeine, Cotinine, Nicotinamide, Nicotine Theophylline
023	80	Male	Pedestrian	Multiple blunt force injuries	Blood, VH, bile	All collected samples	Caffeine, Cotinine, Nicotinamide, Gliclazide

024	27	Male	Pedestrian	Blunt force injuries to chest and abdomen	Blood, VH, bile	All collected samples	Cotinine, Nicotinamide
025	53	Male	Passenger	Blunt force head trauma	Blood, VH, bile	All collected samples	Acetaminophen, Caffeine, Nicotinamide
026	45	Male	Motorcyclist	Multiple blunt force injuries	Blood, VH, bile	All collected samples	Caffeine, Cotinine, Nicotinamide, Zopiclone
027	39	Male	Driver	Consistent with traumatic asphyxia	Blood, VH, bile	All collected samples	Caffeine, Nicotinamide
028	42	Male	Driver	Multiple blunt force injuries to body	Blood, VH, urine	All collected samples	Caffeine, Ketamine, Nicotinamide
029	28	Male	Passenger	Blunt force head injury	Blood, VH, urine	All collected samples	Caffeine, Codeine glucuronide
030	37	Male	Pedestrian	Blunt force head and pelvic injuries	Blood, VH, bile	All collected samples	Caffeine, Methamphetamine, Quinidine, Quinine