Investigating post-mortem redistribution of drugs in a cohort of suspected unnatural deaths in Cape Town, South Africa

Dissertation presented in partial fulfilment of the requirements for the Masters of Medicine (MMed) in Forensic Pathology at the University of Cape Town

By

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Declaration

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Abstract

Introduction: The interpretation of post-mortem toxicological analytical results is complex, specifically, due to the phenomenon of post-mortem redistribution (PMR). The latter needs to be taken into consideration when determining if, and to what extent a drug contributed to death. Additionally, case specific features also play a role and therefore no specific drug concentration can be deemed 'fatal'. The accumulation of drugs in specific body areas before and around death creates drug reservoirs, influencing redistribution after death. However, patterns like concentration ratios between cardiac and peripheral blood aid in understanding PMR tendencies of specific drugs. By contributing data to the larger knowledge pool, we can better understand how different drugs behave in the post-mortem setting, thereby assisting toxicologists and pathologists to come to a rational conclusion regarding the post-mortem toxicological results on a case-by-case basis.

Methods: This study is the first of its kind in South Africa, aiming to investigate the extent of PMR of common drugs of use and misuse. To this end, paired admission femoral blood and autopsy femoral and cardiac blood samples were tested on a quantitative LC-MS/MS panel for of 31 commonly misused drugs.

Results: A total of 109 suspected unnatural cases admitted to the mortuary were included, of which 61 (56%) yielded positive toxicology results. The data was analysed using SPSS Version 28. The most common analytes detected were acetaminophen (n=13; 21.3%), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) (n=20; 37.8%), amphetamine (n=30; 49.2%), methaqualone (n=33; 54.1%) and methamphetamine (n=33; 54.1%). Males represented the majority of cases in the cohort and the mean age of individuals testing positive was 33 years (SD: 10 years).

Cardiac/peripheral (C/P) ratios were calculated and significant pairwise differences with Bonferroni correction were found for amphetamine, methamphetamine and nor-carboxy-tetrahydrocannabinol, corresponding with current literature. Additionally, where the literature was previously lacking data on the PMR of methaqualone, this study suggested that it is less likely to undergo PMR.

Acknowledgements and Format

"It takes a village to raise a child (and to complete an MMed)"....

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Format: Publication-ready format as per Forensic Science International guidelines (Appendix 1)

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Abbreviations and Symbols

°C	degrees Celsius
%	percentage
2-MAPB	(1-(benzofuran-2-yl)-N-methylpropan-2-amine)
4-ANPP	Phenethyl-4-anilino-N-phenethylpiperidine
6-AC	acetylcholine
6-MAM/ACM	6-Monoacetylmorphine
ACE	acetaminophenALP alprazolam
AMI	amitriptyline
AMP	amphetamine
ASB	Academy of Standards Board
BEN	benzoylecgonine
BMI	body mass index
BUP	buprenorphine
C/P	central/peripheral
CBZ	clobazam
CET	cocaethylene
CNS	central nervous system
COC	cocaine
COD	codeine
Conc	Concentration
CRM	certified reference material
CSF	cerebrospinal fluid
CYP	cytochrome P
DIP	diphenhydramine
DZP	diazepam
EME	ecgonine methyl ester
FEN	fentanyl
FTU	Forensic Toxicology Unit
GIT	gastrointestinal tract
HCD	hydrocodone
HMP	hydromorphone
HO-RCS-4	N-hydroxypentyl-RCS-4
HREC	Human Research Ethics Committee
ISO	International Organisation for Standardisation

KET	ketamine
LC-MS/MS	Liquid chromatography tandem mass spectrometry
L/Kg	litre per kilogram
L/P	liver/peripheral
M3G	morphine-3-glucuronide
M6G	morphine-6-glucuronide
MDA	methylenedioxyamphetamine
MDAI	5,6-methylenedioxy-2-aminoindane
MDMA/MDM	3,4-Methylenedioxymethamphetamine
MEC	methcathinone
MEQ	methaqualone
MET	methamphetamine
MOR	morphineMTD methadone
NHA	National Health Act
NPS	new psychoactive substances
ODMV	O-desmethylvenlafaxine
ODT	O-desmethyltramadol
OXC	oxycodone
OXM	oxymorphone
PK-PD	pharmacokinetic-pharmacodynamic
PM	post-mortem
PM/AM	post-mortem/antemortem
PMI	post-mortem interval
PMR	post-mortem redistribution
QSAR	quantitative structure–activity relationship
Reg	regulation
RTA	road traffic accident
SANAS	South African National Accreditation System
sd	standard deviation
SUDA	sudden unexpected death of an adult
THC	tetrahydrocannabinol
THC-COOH/NCT	11-nor-9-carboxy-Δ^9-tetrahydrocannabinol
TIAFT	The International Association of Forensic Toxicologists
TRM	tramadol
UCT	University of Cape Town
Vd	volume of distribution

WCGHW	Western Cape Government Department of Health and Wellness
Yrs	years

Chapter 1 – Introduction and Literature Review

1.1 Introduction

Post-mortem investigations, which include autopsies, are systematic medical investigations of bodies after death (Fitsanakis, 2020). In South Africa, forensic post-mortems are performed by forensic pathologists or forensic medical practitioners and are mandated by The Inquests Act (Act 58 of 1959),which allows for the holding of inquests in cases of death or alleged deaths apparently occurring from other than natural causes (*The Inquests Act, No. 58 of 1959*, 1959). Unnatural deaths are further defined in the Regulations Regarding the Rendering of Forensic Pathology Services (reg 636), in terms of the National Health Act 2003 and the Health Professions Act *(Health Professions Amendment Act, 2007, No. 29 of 2007; National Health Act, No. 61 of 2003; Regulations Regarding the Rendering of Forensic Pathology Service, 2007)*. These include deaths due to direct or indirect physical or chemical influence or arising complications, procedure-related deaths (whether therapeutic, diagnostic or palliative in nature), sudden unexpected deaths, and deaths suspected to be due to an act of omission or commission (*Regulations Regarding the Rendering of Forensic Pathology Service*, 2007).

Forensic toxicology, an ancillary component of the death investigation, aims to determine potential drug or chemical toxicity or impairment prior to death and assess its contribution to the cause of death. Forensic pathologists may request toxicological analyses for various substances including ethanol, scheduled medicinal and recreational drugs, and chemicals, as these may be involved in a myriad of fatal incidents, including, but not limited to motor vehicle accidents, workplace accidents, or overdoses. The results of these tests are of great importance in medicolegal investigations, not only to confirm or exclude fatal intoxication, but also to be able to estimate the degree of incapacitation at the time of death (Zilg et al., 2017). In the living, reference values are available for therapeutic, and toxic levels of most drugs in serum/plasma, however, these rarely apply in the post-mortem setting (Pelissier-Alicot et al., 2003).

It has been well established that drug concentrations at the time of autopsy do not necessarily reflect those at the time of death. This complicates the interpretation and conclusion of cause of death in forensic cases where drugs are involved (Pelissier-Alicot et al., 2003). Castle et al. (2022) re-iterate the wide overlap of therapeutic and potentially fatal blood concentrations with certain drugs, such as opioids (Castle et al., 2022).There are several factors that contribute to the difference in drug concentrations and the complication of interpretation, including drug instability, lack of specimen integrity and site of collection, and post-mortem redistribution (PMR) of the drugs. PMR is a phenomenon that involves site- and time-dependent changes of drug concentrations in a body after death (Mantinieks et al., 2021). Despite the common use of post-mortem concentrations to calculate or assume antemortem concentrations for certain drugs such as ethanol, estimating the ante-mortem concentration of a drug from a post-mortem blood drug concentration is not

recommended, due to the myriad of factors that may influence the concentrations. Providing pathologists and toxicologists with more insight into PMR for certain drugs, together with stability and other interpretive data, may assist in arriving at a rational interpretation of post-mortem toxicological results within the context of the death (McIntyre, I., 2016).

1.2 Background / Literature review

1.2.1. Post-mortem redistribution

The human body is not a static entity after death (Chesser et al., 2019). PMR is a prime example of this, as it encompasses the site- and time-dependent variations in drug concentrations in biological specimens after death (Mantinieks et al., 2021). This complex phenomenon is well recognised, yet under-explored and it complicates the interpretation of drug concentrations in decedents in medicolegal investigations (Saar et al., 2012). There is a definite danger of over- or under interpretation of post-mortem drug levels, for example an artefactually elevated post-mortem drug level may be misinterpreted as a lethal overdose (Pounder & Jones, 1990). The opposite may also be true, where falsely low levels may cause the contribution of drugs to the death to be overlooked or underestimated. Castle et al., (2022) re-iterated the importance of obtaining drug use history, as there is a wide overlap of therapeutic and potentially fatal blood concentrations, which may be attributed to the repeated administration of drugs such as opioids, leading to tolerance (Castle et al., 2022).

Many avenues have been investigated in order to better understand a drug's propensity for undergoing PMR and in doing so improve interpretation of post-mortem toxicology results. These include drug concentration ratios between cardiac and peripheral blood (C/P) (Emaus et al., 2023). A liver-to-peripheral (L/P) ratio has also been explored as an indicator for PMR (Lemaire et al., 2017), as drug blood-brain concentrations (Nedahl, Johansen & Linnet, 2021). Despite PMR playing an important part of the medicolegal death investigation, there are currently no accepted, reliable methods for the determination of PMR, due to its complexity and the numerous factors and mechanisms, which may cause PMR (Brockbals et al., 2021).

1.2.2. Mechanisms of PMR

The underlying mechanisms of PMR are complex and may involve various processes, such as diffusion, cell breakdown and other post-mortem processes, all of which have inter-individual variation (Yarema & Becker, 2005). Post-mortem drug levels may be falsely low due to tissue uptake, metabolism, or instability of the analyte(s). Alternatively, the levels may be falsely high due to passive release of drugs from high concentration reservoirs, down a concentration gradient (Pelissier-Alicot et al., 2003), or post-mortem synthesis. The diffusion can occur through blood vessels or be transparietal, from the lung into nearby vasculature. As a result

of such high concentration reservoirs, there may be variations in drug concentrations in the same matrix (e.g., blood) from the same case, depending on the site of sampling and its proximity to high concentration reservoirs. For example, some sites, such as the heart are in close proximity to high concentrations of analytes in the gastrointestinal tract (Pelissier-Alicot et al., 2003). This gives rise to potential diffusion into cardiac blood, leading to artefactually high concentrations.

Further causes of PMR include post-mortem processes such as putrefactive changes, cell death, coagulation of blood and hypostasis (Pelissier-Alicot et al., 2003). Post-mortem degradation of cell membranes allows for diffusion of elements from one tissue to another, altering the drug concentrations (Gerostamoulos et al., 2012). Post-mortem microbial action may further degrade drugs and metabolites, further complicating the interpretation of concentrations. (Castle et al., 2022).

Another reason one cannot utilise living patient reference values for therapeutic, and toxic concentrations in the post-mortem setting in isolation, is due to the fact that that the composition of post-mortem blood is different. Usually whole blood is analysed in a post-mortem setting, whereas plasma and serum are analysed in ante-mortem cases, and the distribution of drugs between these matrices may vary (Pounder & Jones, 1990). In addition to decomposition, the condition and position in which a body was found, transported and stored can cause changes to drug concentration (Gerostamoulos et al., 2012). The position of a corpse can influence the concentrations in certain areas, with corpses found in a left side position having lower cardiac-to-peripheral (C/P) ratios when compared to those in a right sided position. This may be due to hypostasis, post-mortem 'reflux' and gravitational pull (Kamphuis et al., 2021).

Not only do all these factors influence PMR, but the properties of individual analytes including lipophilicity, volume of distribution, residual metabolic activity, acidic/basic properties and protein binding affinity also play a role (Lemaire et al., 2017). Additional drug specific factors influencing PMR, include the route of administration, bioavailability, synergism and antagonism of various drugs (Chung & Choe, 2019), additionally, the metabolic phase of the specific analyte at the time of death may also influence PMR, for example high arterial concentrations of diazepam and ethanol during the absorption phase, and higher venous concentrations during the elimination phase of drugs such as furosemide and propranolol (Pelissier-Alicot et al., 2003). Once a sample is collected, further factors may influence the interpretation of post-mortem toxicology results include variations in drug stability, storage conditions and time between collection and testing (Kintz et al., 2020).

1.2.3. Sources of PMR

Drugs may sequester in the ante-mortem and peri-mortem stage into organs and tissues, known as drug reservoirs (Pelissier-Alicot et al., 2003)(Figure 1). These include hollow organs such as the gastrointestinal tract (GIT), especially the stomach, which can contain undigested and unabsorbed drugs, and organs that function to concentrate drugs, such as the liver, lungs, heart, and in some cases, the renal cortex (Pounder & Jones, 1990).

Redistribution from the stomach may occur rapidly through blood vessels to the left cardiac chambers, aorta, right cardiac chambers, and inferior vena cava, often within hours after death for substances like tricyclic antidepressants (Pelissier-Alicot et al., 2003). Stomach contents can be regurgitated or shifted during the post-mortem period into the airways, leading to redistribution of drugs into pulmonary vessels and subsequently into the heart blood.

The lungs can accumulate drugs because they receive a large volume of blood from the right ventricle of the heart. After death, drugs can be redistributed from the lungs to the cardiac chambers and vessels in the thorax (Pelissier-Alicot et al., 2003). Additionally, the fluid in the pleural and peritoneal cavities assists in the movement of drugs between organs and the different body cavities (Hilberg, Mørland & Bjørneboe, 1994).

Drugs can be further redistributed from the liver, located in the peritoneal cavity, into the inferior vena cava via the hepatic veins and end up in organs in other body cavities, such as the right cardiac chambers, pulmonary blood vessels, and/or peripheral blood (Pounder, 1993).The anatomical position of the liver lends itself to direct redistribution of drugs into adjacent organs, including the stomach, proximal duodenum, and gallbladder (Pounder, 1993).

Cardiac drugs such as digoxin, calcium channel blockers, and quinidine are concentrated in the heart muscle of patients in vivo, leading to concentrations up to 30 times higher compared to the blood within the heart (Pelissier-Alicot et al., 2003). After death, the drugs can diffuse from the myocardium back into the heart blood and subclavian blood vessels, altering the concentrations at these sites. Subclavian blood cannot be considered a peripheral specimen in such cases (Prouty & Anderson, 1990).

Considering that the stomach, lungs, and heart can be seen as drug sequesters, they may allow drugs to move back into the blood system in the central regions. It is for the above reasons that peripheral blood specimens are preferred for quantitative interpretation, given that peripheral sites are less likely to be affected by PMR, due to their proximity to these organs and reservoirs.

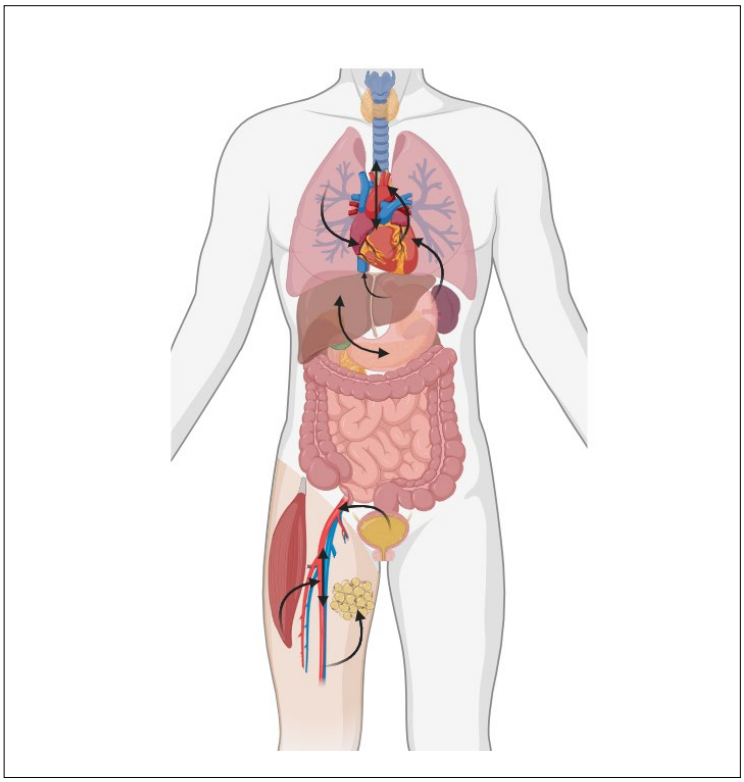

Figure 1: Possible post-mortem diffusion routes of drugs from high concentration reservoirs into blood sources in the body.

1.2.4. Site-dependent PMR variations

Biological samples for toxicological analysis may be obtained from many sites or tissues in the body, and the collection of these may itself alter the validity of the result obtained (Pounder & Jones, 1990). High concentrations in certain regions can be linked to the route of administration – such as smoking/inhalation leading to high pulmonary concentrations (Mueller et al., 2021). A study performed in Sweden has shown that drug concentrations in central blood were generally higher than the concentrations in peripheral blood. Specific drugs which exhibited this, included Oxazepam, Metoprolol, Warfarin, Diazepam, Nordazepam, Pregabalin, Sertraline, Desmetylsertraline, EDDP, Alprazolam, Propiomazine, Methadone, Paracetamol, Lamotrigine, Tramadol, O-desmetyltramadol, Zopiclone, Citalopram, Duloxetine, Metoprolol, Alimemazine, Morphine, Codeine, Oxycodone, MDMA, MDA, Salicylic acid, Amitriptyline, Nortriptyline, Olanzapine and Venlafaxine. (Zilg et al., 2017). In addition, the drug concentrations also varied between arterial and venous blood, when comparing blood from the carotid artery to the jugular vein, the former having higher concentrations (Zilg et al., 2017). Statistically significant increases in drug concentrations were found in blood from the right heart (central), compared to the external iliac vein (peripheral) (Zilg et al., 2017). This study also showed that there was no significant difference in drug concentrations in drugs with low volume of distribution and cases with a short post-mortem interval (PMI). The latter is another important factor in PMR. Significant differences in concentration were noted with longer time periods between death and the autopsy

and collection of biological samples (Zilg et al., 2017). Other studies have reviewed variation in central sites – heart and subclavian vessels - and others at peripheral sites – iliac, femoral, and popliteal vessels (Lemaire et al., 2017). They also have important comparative findings and recommend popliteal venous blood sampling as it appears to be more resistant to processes such as PMR (Lemaire et al., 2017).

Examples of these site-dependent concentration differences were highlighted in a study evaluating free morphine and total morphine, which were higher than the peripheral concentrations, when comparing femoral vein and femoral artery blood to cardiac blood, when investigating suspected heroin overdoses (Crandall et al., 2006). Mean concentrations of drugs such as morphine, methadone and their respective metabolites tend to be lower at peripheral sites compared to cardiac concentrations (Lemaire et al., 2017). The same study revealed that the concentration of diazepam and its metabolites were slightly higher in peripheral samples, compared to the cardiac concentrations (Lemaire et al., 2017).

1.2.5. Markers of PMR

In light of interpretive difficulties in post-mortem toxicology, including the site-dependent variations of drug concentrations included in PMR, some authors have explored the use of alternative strategies to interpret PMR, such as the use of concentration ratios of drug at different anatomical sites. Concentration ratios between cardiac and femoral blood (C/P ratio) is often used to determine a drug's propensity for PMR, as central blood is more prone to these changes. Peripheral blood should not be collected via blind stick method, but rather via direct visualisation and collection from a ligated peripheral vein, therefore functionally isolated from central contamination (Lemaire et al., 2017) without milking the vessel, whereas cardiac blood should be sampled from the right atrium or the inferior vena cava (Stimpfl et al., 2008) A C/P ratio less than or equal to 1 suggests the absence of PMR, although there are some exceptions, such as codeine (Brockbals et al., 2018). Therefore a liver-to-peripheral ratio has also been explored as an indicator for PMR (Lemaire et al., 2017). Liver tissue is invaluable in toxicological analysis, as it is the site of metabolism for most drugs and will therefore contain both the parent drug and its metabolites. Ideally, the liver sample should be harvested from deep within the right lobe (Stimpfl et al., 2008). Nedahl et al. 2021, have suggested the supplementary use of blood-brain concentrations for the interpretation of stimulants, particularly in cases of exsanguination, decomposition and due to the brain's lack of esterases which degrade cocaine (Nedahl, Johansen & Linnet, 2021). Steuer (2018) summarised the main parameters to determine a high propensity for PMR as follows: C/P ratio of >1, L/P ratio of >20-30 and volume of distribution (Vd) of >3 (Steuer, 2018).

Other solutions such as the use of endogenous molecules, as a surrogate marker for PMR, have been explored and found to be valuable in assessing PMR of xenobiotics with central actions (Brockbals et al., 2021). The same authors have found statistically significant correlations between morphine/methadone and several

endogenous compounds/features, including creatinine, glutaric acid, hypoxanthine, fructose, alanine, and three fatty acids (pentadecanoic acid, palmitoleic acid, and linoleic acid) (Brockbals et al., 2020).

Iskiera et. al. (2021) investigated the correlation of xenobiotic concentrations between typical specimens, such as blood, urine and vitreous humor, as well as bone marrow. Their research showed strong correlations between the concentrations found in blood or vitreous humor and those found in bone marrow, with the exceptions of diazepam and 7-aminoclozepam. However, despite the usefulness of bone marrow as an alternative matrix, the PMR in this regards is poorly understood, further highlighting the need for future studies (Iskierka et al., 2021). Another example of the usefulness of alternative matrices in post-mortem toxicology is the fact that some drugs, such as new psychoactive compounds and alcohols have a site of action in the central nervous system (CNS), making cerebrospinal fluid (CSF) a potentially important sample (Wachholz, Skowronek & Pawlas, 2021).

1.2.6. Time-dependent PMR variations

In the setting of medicolegal investigations, post-mortem interval (PMI) is the time period between death and the performance of the autopsy (Hayman, 2021). PMR processes progress with time (Lemaire et al., 2017). A study on the PMR of antipsychotic drugs, conducted in Australia, assessed cases with PMI of up to nine (9) days (Saar et al., 2012). They found that the average increase in blood concentrations after admission ranged up to 112% for drugs such as chlorpromazine and olanzapine but decreases in concentration of up to -43% were also documented for 9-OH-risperidone (Saar et al., 2012). Significant variations existed among sample pairs, with considerable standard deviations and notable day-to-day fluctuations. These variations underscore the challenge in interpreting post-mortem drug concentrations and the need for decreased PMI. (Saar et al., 2012).

A study reviewing the ratio of post-mortem femoral blood drug concentrations and antemortem (specimens from hospitals include whole blood, serum or plasma) concentrations of antidepressants calculated the ratio to be >1 and those for benzodiazepines as <1 (Mantinieks et al., 2021). These findings were further consolidated by a study performed in Switzerland, which additionally identified minimal median post-mortem changes for drugs such as morphine and codeine (Brockbals et al., 2021). An Australian study compared femoral blood samples taken at admission to the mortuary, to those taken at autopsy, with PMI varying between 0.5 to 164 hours (6.4 days) (Gerostamoulos et al., 2012). The authors calculated increases in drug concentrations ranging from 30% (citalopram, mirtazapine and sertraline) up to 300% (doxylamine) (Gerostamoulos et al., 2012). This suggests that more reliable results are obtained by minimising the PMI, but also that the concentration may be altered in both directions - positively and negatively. Statistically significant pre-autopsy concentration increases were identified for methadone, EDDP, fluoxetine, mirtazapine and

sertraline (Gerostamoulos et al., 2012). Other agonal and cadaveric phenomena which can lead to concentration changes include cell autolysis and decomposition and the influence of bacteria (Sastre et al., 2017). All these factors need to be taken into consideration when interpreting post-mortem toxicology results.

1.2.7. Case specific factors influencing PMR

Patient specific factors may influence drug reservoirs, drug concentrations and drug metabolism. These include factors such as bariatric surgery, underlying diseases, co-administration of drugs, cytochrome polymorphisms, body mass index (BMI), trauma and resuscitation. Bariatric surgery can alter the individual's pharmacokinetic abilities because it influences the gastrointestinal uptake and/or metabolism of drugs. In so-called 'malabsorptive procedures' this occurs due to a decrease in functional gastrointestinal length, a faster intestinal transit time, a reduction in the absorptive surface area, decreased blood flow and a reduction in bile acid mixing, while increasing the intestinal pH (Bishop-Freeman et al., 2019). Additionally, comorbidities, such as chronic liver or kidney diseases, must be taken into consideration as it may, for example, lead to greater post-mortem drugs increases, such as in the case of fentanyl (Reiter et al., 2019). Impaired kidney function may lead to a reduction in drug clearance while hepatic impairment can interfere with drug metabolism. These conditions may therefore lead to an increased accumulation of the lipophilic non-metabolised or non-eliminated fentanyl in the fatty tissue *in vivo* (Reiter et al., 2019).

A rat model study revealed that co-administration of substances such as alcohol, can influence PMR, such as for MDMA. The underlying mechanism for this is suggested to be an alcohol-induced accelerated diffusion of the MDMA from the stomach (Liang et al., 2017). Co-administration of certain medications such as ritonavir and fluconazole, which can inhibit metabolic liver enzymes, can lead to reduced metabolism of drugs such as fentanyl (Geile et al., 2019). Similarly, underlying liver conditions such as polymorphisms of metabolic enzyme cytochrome P450 2D6 (CYP2D6) may influence metabolism and PMR (Matsusue et al., 2018). Since some drugs are distributed into fat, the BMI of a decedent can also play a role in PMR, for example, cocaine has a higher lipophilicity than EME, causing the former to have larger reservoirs in adipose tissue, which may influence PMR (Emaus et al., 2023).

Additional patient specific factors such as trauma**,** can cause damage to the physical epidermal barrier, allowing an influx of microorganisms which can influence the concentrations of cocaine and EME, by means of their enzymatic activity, adding to post-mortem drug alteration and/or degradation (Emaus et al., 2023). Additionally, resuscitation may alter drug concentrations and lead to higher C/P ratios. The latter is thought to be due to the resuscitative movements allowing increased diffusion to cardiac blood or internal injuries (Kamphuis et al., 2021).

1.2.8. Study Rationale

The phenomenon of PMR of drugs is an important aspect in the field of forensic toxicology, particularly in the interpretation of drug concentrations obtained post-mortem. Despite its recognised impact, there is a notable lack of research on PMR in the South African context. This gap in knowledge is particularly critical given the unique drug misuse patterns prevalent in South Africa, particularly in relation to methaqualone, methamphetamine, and amphetamine. To address this, we initiated a study in Cape Town, focusing on the post-mortem redistribution of several key analytes commonly misused in the population, with the aim of assessing changes in drug concentrations from the time of admission (femoral blood samples) to the autopsy (cardiac and peripheral blood samples) in a series of suspected unnatural deaths. The rationale for targeting the specific drugs is based on an internal study performed by the Division of Clinical Pharmacology at the University of Cape Town. For this study, a total of 29 drugs and metabolites were selected for the panel. The method was designed to detect and quantify selected drugs of abuse that are frequently encountered in forensic casework. The testing panel included multiple drug classes and analytes that were selected based on the following considerations:

The recommendations by the Academy Standards Board (ASB) for the analytical scope and sensitivity of forensic toxicological testing in medico-legal death investigations; the frequency of detection in post-mortem casework [(based on evaluation of toxicology results received from the Forensic Chemistry Laboratory (2018 to 2020) and UCT Division of Clinical Pharmacology (2012 to 2021)]; and the availability of certified reference materials (CRM).

This research serves as a valuable resource for forensic pathologists and toxicologists working in the region (and globally) as well as provide a framework for future local studies in this area.

Chapter 2: Publication-Ready Manuscript

Abstract

Introduction: The interpretation of post-mortem toxicological analytical results often poses great difficulty, particularly due to the phenomenon of post-mortem redistribution (PMR). PMR involves the site- and time-dependent variations in drug concentrations in biological specimens after death. The effects of PMR need to be taken into consideration when determining if, and to what extent a drug contributed to death. Additionally, case specific features also play a role (and therefore no specific drug concentration can be deemed 'fatal'. By contributing data to the larger knowledge pool, we can better understand how different drugs behave in the post-mortem setting, thereby assisting toxicologists and pathologists to come to a rational conclusion regarding the post-mortem toxicological results on a case-by-case basis.

Methods: This study is the first of its kind in South Africa, aiming to investigate the extent of PMR of common drugs of use and misuse. To this end, paired admission femoral blood and autopsy femoral and cardiac blood samples were tested on a quantitative UPLC-MS/MS panel for of 31 commonly misused drugs

Results and Discussion: A total of 109 suspected unnatural cases admitted to the mortuary were included, of which 61 (56%) yielded positive toxicology results. Cardiac/peripheral (C/P) ratios were calculated and significant pairwise differences with Bonferroni correction were found for amphetamine, methamphetamine and nor-carboxy-tetrahydrocannabinol, corresponding with current literature. Additionally, where the literature was previously lacking data on the PMR of methaqualone, this study suggested that it is less likely to undergo PMR.

Keywords*:* C/P ratio, Forensic Toxicology, Pharmacokinetics, PMR, Post-mortem, Redistribution,

1. Introduction

It is well established that drug concentrations at the time of an individual's autopsy do not necessarily reflect those at the time of their death, which complicates the interpretation and conclusion of cause of death in forensic cases where drugs are involved (Pelissier-Alicot et al., 2003). Post-mortem redistribution (PMR) is a phenomenon that involves site- and time-dependent changes of drug concentrations in a body after death. Estimating the dose administered and/or concentration of a drug in blood prior to death using post-mortem blood drug concentrations is not recommended, due to the post-mortem artefacts that may alter concentrations after death (McIntyre, I., 2016). Providing pathologists and toxicologists with more insight into PMR for certain drugs, together with stability and other interpretive data, may assist in coming to a rational interpretation of post-mortem toxicological results within the context of a death.

The mechanisms underlying PMR are multifaceted, encompassing diffusion, tissue and cell autolysis, and individual variations (Yarema & Becker, 2005). Post-mortem drug levels can change over time, appearing either lower due to tissue uptake and metabolism or higher due to passive drug release from reservoirs. Diffusion through blood vessels or transparietal routes may contribute to variable drug concentrations within the same matrix, influenced by proximity to high-concentration reservoirs (Pelissier-Alicot et al., 2003). PMR is also influenced by post-mortem changes like putrefaction, cell breakdown, coagulation, and hypostasis, allowing elements to diffuse across tissues and alter drug concentrations (Pelissier-Alicot et al., 2003). Post-mortem microbial action may degrade drugs or generate unusual products (Castle et al., 2022). Utilising clinical reference values for post-mortem toxicology is not advised, due to differences in blood composition during life and post-mortem (whole blood vs. plasma/serum) (Pounder & Jones, 1990). Other factors which may influence the drug concentrations include decomposition, post-mortem body positioning, and storage conditions (Gerostamoulos et al., 2012). Individual drug properties, metabolic phases, and factors such as route of drug administration, bioavailability, and synergism further contribute to PMR (Chung & Choe, 2019). Interpretation of post-mortem toxicology results is further complicated by drug stability, storage conditions of specimens, and time elapsed between collection and testing (Kintz et al., 2020).

While post-mortem drug concentrations cannot be equated to ante-mortem levels due to factors described, they may exhibit discernible patterns that aid in interpretation. These patterns, albeit influenced by post-mortem changes and redistribution, can still offer valuable insights for understanding the presence, behaviour, and potential effects of substances in the deceased individual (Abdelaal et al., 2023). An example of such a pattern is the use of concentration ratios of drug concentrations at different anatomical sites. Concentration ratios between cardiac and peripheral (usually femoral) blood (C/P ratio) is often used to determine a drug's propensity for PMR, as central blood is more prone to these changes (Abdelaal et al., 2023). It is generally accepted that the majority of drugs with a C/P ratio of >1 have a propensity for PMR (Steuer, 2018). The post-mortem interval (PMI), defined in this setting as the time between death and autopsy, must also be taken into consideration (Hayman, 2021). During the post-mortem interval, drug concentration may be altered in both directions - positively and negatively - by the various post-mortem processes. This was established by analysing paired blood samples from 149 cases, in which 30 different substances were detected. (Gerostamoulos et al., 2012). Although these changes may be insignificant for some drugs and unrelated to the length of PMI, the concentrations of some drugs, such as 6-acetylmorphine, caffeine and 9-hydroxy-risperidone, have shown to decrease significantly (Gerostamoulos et al., 2012). Where possible, it is recommended to minimise factors within one's control, such as the PMI, to reduce the likelihood of variation in drug concentrations over time, particularly with drugs mentioned above.

An Australian study highlighted the complexity of interpreting PMR, which was illustrated by the findings of amphetamine, MDMA, and MDA. In the study, paired blood samples from 811 coronial cases were analysed, and 42 drugs and drug metabolites were identified. MDMA, and MDA showed to have a lower propensity for PMR (Mantinieks et al., 2021). In contrast, a 10-year Dutch study, performed by de Groot et al. (2023), focusing mainly on amphetamines (n=112) and benzodiazepines (n=179), reported low amphetamine C/P ratios in cases with high concentrations of amphetamine in femoral blood. The latter may be due to a short time between administration and death, thus limiting distribution of the drug into reservoirs (de Groot et al., 2023). These authors also found that C/P ratios of methylenedioxyamphetamine (MDA) were significantly lower in cases where there were resuscitation attempts, and higher when the cause of death was trauma related (de Groot et al., 2023). The authors thus recommended that cause of death, together with femoral blood concentrations should be taken into account in assessing PMR.

A recent study on cannabinoids, performed by Swiss and Canadian authors compiled a dataset comprised of 276 post-mortem cases and 351 antemortem cases, over a two-year period, has revealed that post-mortem blood concentrations of THC were significantly higher than in antemortem blood. Additionally, they showed that THC has a tendency to redistribute towards peripheral blood, whereas THCCOOH redistributes towards central blood. The authors advise that PMR can be suspected in THC, if there is a high THC blood concentration, the C/P ratio is less than 1.0, when the blood THC/THCCOOH ratio is more than 1.0 and THCCOOH is not detectable in urine (Tascon et al., 2023).

To assist pathologists and toxicologists in a local South African setting, we performed a pilot study to investigate statistical changes in the admission femoral blood, autopsy cardiac, and autopsy peripheral blood concentrations of frequently misused drugs (with a focus on methamphetamine, amphetamine, and methaqualone), in a series of suspected unnatural death cases in Cape Town, South Africa.

2. Materials and Methods

2.1 Study Setting and Inclusion Criteria

Biological specimens (whole blood) were collected from suspected unnatural deaths admitted to Salt River Mortuary, in the Cape Town metropole, over a five-month period (February - June 2023). Salt River Mortuary is one of 16 forensic mortuaries in the Western Cape Province and services the west metropole of the City of Cape Town. Cape Town has an estimated population of 4,602,248, with approximately 69% of these aged between 15 and 65 years (Western Cape Government, 2020). Salt River Mortuary admits ±4,000 suspected unnatural death cases annually. Approximately 60% of the total admissions to the Western Cape Forensic Pathology services are due to injuries, classified into the broad groups of homicide, suicide and accidents (Evans et al., 2018).

Cases were selected by the researcher (forensic pathology registrar) on admission to the mortuary and included suspected unnatural deaths where whole blood for toxicological analysis was to be routinely collected for ancillary investigations (i.e., homicide, suicide, accidental and sudden unexpected deaths). Exclusion criteria included decedents below the age of 18 years, decedents with significant signs of decomposition, cases where the date of death was not known with reasonable certainty, cases where adequate samples could not be obtained (exsanguination or extensive burning or trauma), cases with features of terminal resuscitative efforts (including intravenous fluid administration), and cases where there was prolonged hospital admission prior to death.

2.2 Specimen Collection

The researcher (forensic pathologist) was stationed at the mortuary facility for the admission of decedents and selection for their inclusion to the study. Pre-prepared study packs were utilised, comprised of an admission specimen toxicology request form with a 4 mL grey-top tube vacutainer (containing sodium fluoride and potassium oxalate) labelled as 'F1' (admission femoral blood), and a separate post-mortem specimen test request form along with two 4 mL grey-top tube vacutainers, labelled as 'F2' (autopsy femoral blood) and 'C' (autopsy cardiac blood), respectively. All request forms and vacutainers were labelled with a unique study number, the sample (F1/F2/C), date- and time of collection and the unique Salt River Mortuary case number.

Upon admission to the mortuary, and following confirmation that the case met the required inclusion criteria, the researcher collected an admission whole blood sample (F1) from the decedent. These samples were obtained using a sterile needle and syringe, through percutaneous venepuncture (blind-stick technique) in the right or left femoral region (based on blood availability and injury location) of the decedent. Each sample consisted of 3-4 ml of whole blood and was placed in vacutainers containing sodium fluoride and potassium oxalate for preservation. The pre-populated request form and vacutainers were attached to the case paperwork handed to the pathologist to whom the case was allocated and a note was made on the case docket folder to indicate that the team assisting the family with identification should inform the researcher when the next-of-kin arrived at the mortuary, to obtain informed consent.

Autopsy specimens, specifically femoral blood (F2) and cardiac blood (C), were collected by the forensic pathologist performing the autopsy and/or by a supervised forensic pathology officer, who assisted with evisceration. The autopsies performed ranged from full autopsies, where the skull-, chest-, abdominal- and pelvic cavities were opened, the organs removed and individually examined, to partial autopsies, where only some of the cavities are opened to examine targeted organs, based on the discretion of the pathologist. The latter is usually reserved for cases where a natural cause of death is identified and, therefore did not require further examination, whereas the former may include further tests, including blood alcohol concentrations,

toxicological analysis and histology, depending on the case. The femoral blood was obtained from either the left or right leg, through an incision through the skin and subcutaneous tissue of the superomedial thigh, visualisation and transection of the femoral blood vessels (cut-down procedure) followed by aspiration with a sterile syringe. Cardiac blood was directly aspirated from the right atrium or ventricle of the heart, depending on blood availability, predominantly after opening the pericardial sac, using a clean needle and syringe. In a minority of cases, where blood volume was minimal, blood was aspirated directly from the chamber during dissection of the organ. Similar to the admission samples, these autopsy samples also contained 3-4 ml of blood and were preserved in vacutainers containing sodium fluoride and potassium oxalate. In the few cases where the autopsy pathologist did not indicate the time of specimen collection, the time of collection was estimated, based on the usual autopsy times at the mortuary.

All collected specimens were promptly refrigerated (4 °C) following collection. On a daily basis, the specimens were transported from the mortuary to the Forensic Toxicology Unit (FTU) laboratory, where they were frozen at -20°C to maintain their integrity until the time of testing. Specimens obtained outside regular working hours and over weekends were immediately transported to the laboratory and placed in the freezer to ensure preservation.

2.3 Toxicological Analysis

Specimens were submitted for toxicological analysis with the Western Cape Government Department of Health and Wellness (WCGHW) Forensic Toxicology Unit (FTU) laboratory in Observatory, Cape Town. The specimens were prepared directly for targeted quantitative analyses, which was performed by forensic toxicologists, using a Waters® ACQUITY I-Class UPLC coupled to a XEVO TQD liquid chromatography tandem mass spectrometer system (UPLC-MS/MS). The analytes included in the targeted quantitation are indicated in Table 1. The method was previously validated in accordance with the recommendations of the Academy Standards Board (ANSI/ASB) Standard 036 and South African National Accreditation System (SANAS) TG 41-03.

Table 1: Analytes and internal standards included in the targeted quantitative UPLC-MS/MS analysis with the lower limits of quantitation in brackets (ng/mL)

Analyte	Corresponding ISTD [a]	Analyte	Corresponding ISTD*	Analyte	Corresponding ISTD*
6-Acetylmorphine (10)	6-Acetylmorphine-d3	Codeine (10)	Codeine-d3	Methamphetamine (20)	Methamphetamine-d5
Acetaminophen (100)	Acetaminophen-d4	Diazepam (20)	Diazepam-d5	Methaqualone (50)	Methaqualone-d7
Alprazolam (5)	Alprazolam-d5	Diphenhydramine (40)	Diphenhydramine-d3	Methcathinone (20)	Methamphetamine-d5
Amitriptyline (20)	Amitriptyline-d3	Fentanyl (1)	Fentanyl-d5	Morphine (10)	Morphine-d3
Amphetamine (20)	Amphetamine-d6	Hydrocodone (10)	Hydrocodone-d3	O-desmethyltramadol (10)	O-desmethyltramadol-d6
Benzoylecgonine (20)	Benzoylecgonine-d3	Hydromorphone (10)	Hydromorphone-d3	Oxycodone (10)	Oxycodone-d3
Buprenorphine (4)	Buprenorphine-d4	Ketamine (20)	Ketamine-d4	Oxymorphone (10)	Oxymorphone-d3
Clobazam (10)	Diazepam-d5	MDA [b] (10)	MDA-d5	THC-COOH (10)	THC-COOH-d3
Cocaethylene (20)	Cocaethylene-d3	MDMA [c] (10)	MDMA-d5	Tramadol (20)	Tramadol-d3
Cocaine (20)	Cocaine-d3	Methadone (20)	Methadone-d3		

[a] ISTD: Internal standard; [b] MDA: methylenedioxyamphetamine; [c] MDMA: methylenedioxymethamphetamine; [d] THC-COOH: delta-9-nor-carboxy-tetrahydrocannabinol

Blood samples were extracted, and qualitative analysis was performed by the FTU analysts using Ostro™ Pass-Through Sample Preparation plates (Waters Corporation, Milford, MA, USA). Briefly, 150 µL of lysis buffer (0.1 M $ZnSO_4$/0.1 M $NH_4CH_3CO_2$) was added to each well of the plate, followed by 50 µL of blood, and then 600 µL of acetonitrile (ACN) containing 0.1% formic acid and internal standards (final concentration of 60 ng/mL). The Ostro™ plate was vortexed and a positive pressure manifold (Waters, Milford, MA, USA) was used to elute the samples, which were evaporated to dryness, and reconstituted in 50 µL of equal parts of 2% ACN and 1% formic acid, as well as 5% ammonium hydroxide (NH4OH) in ACN:methanol (1:1) and vortexed thoroughly. The supernatants were transferred to HPLC vials which were loaded onto the autosampler for injection (5 µL into the UPLC-MS/MS using a ACQUITY BEH C18 column (130 Å, 1.7 µm particle size, 100 mm x 2.1 mm I.D.) fitted with an ACQUITY UPLC BEH C18 VanGuard pre-column (130 Å, 1.7 µm particle size., 5 mm x 2.1 mm I.D.) (Waters, Milford, MA, USA). The instrumental conditions are outlined in table 2 and the multiple reaction monitoring (MRM) parameters for analytes and internal standards for whole blood method are indicated in table 3. The laboratory does not run external quality controls batch-on-batch, however proficiency testing samples are run according to the laboratory's internal proficiency testing plan and schedule as per the standard proficiency testing requirements. This is to ensure and monitor the validity of the laboratory's test results according to ISO 17025:2017. Analysis of the instrumental data was carried out using MassLynx software v4.2.

Table 2: Instrumental conditions

Column temperature	40 ˚C		**Ionisation mode**	Electrospray ionisation positive mode
Sample temperature	10 ˚C			
Flow rate	0.6 mL/min		**Acquisition mode**	Multiple Reaction Monitoring
Injection volume	5 µL			
Run time	7 min		**Source temperature**	150 ˚C
Mobile phase	(A) 0.1% Formic acid in water (B) 0.1% Formic acid in acetonitrile			
Gradient			**Desolvation temperature**	400 ˚C
Time (min)	**% Mobile Phase A**	**% Mobile Phase B**		
0:00	98	2	**Desolvation gas flow**	850 L/hr
3:33	33	67		
4:00	10	90	**Cone gas flow**	10 L/hr
5:50	10	90		
6:00	98	2	**Capillary voltage**	2.5 kV
7:00	98	2		

Table 3: MRM parameters for analytes and internal standards for whole blood method

#	Analyte	Precursor ion (m/z)	Product ions (m/z)		DT (secs)	CV (V)	CE (eV)		RT (min)
1	6-acetylmorphine	328.2	165.1	211.1	0.006	52	35	25	1.31
2	6-acetylmorphine-d3	331.1	165.0	-	0.006	45	50	-	1.30
3	Acetaminophen	152.0	110.1	93.2	0.007	40	22	20	1.08
4	Acetaminophen-d4	156.2	114.1	-	0.007	30	15	-	1.08
5	Alprazolam	309.0	205.0	281.1	0.024	50	43	30	2.75
6	Alprazolam-d5	314.1	210.1	-	0.024	60	42	-	2.74
7	Amitriptyline	278.2	91.1	105.1	0.024	44	20	24	2.56
8	Amitriptyline-d3	281.3	91.1	-	0.024	38	30	-	2.57
9	Amphetamine	136.2	119.1	91.1	0.006	25	10	20	1.31
10	Amphetamine-d6	142.0	93.1	-	0.006	36	26	-	1.30
11	Benzoylecgonine	290.1	168.1	105.0	0.006	36	18	36	1.55
12	Benzoylecgonine-d3	293.1	171.1	-	0.006	78	22	-	1.55
13	Buprenorphine	468.2	101.0	396.2	0.024	60	42	38	2.30
14	Buprenorphine-d4	472.3	59.1	-	0.024	96	48	-	2.30
15	Clobazam	301.0	259.0	224.0	0.031	40	18	32	2.99
16	Cocaethylene	318.2	196.2	82.1	0.024	42	20	30	2.04
17	Cocaethylene-d3	321.2	85.2	-	0.024	80	34	-	2.04
18	Cocaine	304.1	182.1	82.1	0.034	40	18	28	1.84
19	Cocaine-d3	307.2	185.2	-	0.034	42	20	-	1.84
20	Codeine	300.1	215.2	165.1	0.006	54	26	38	1.20
21	Codeine-d3	303.0	215.0	-	0.006	50	26	-	1.20
22	Diazepam	285.1	154.0	193.1	0.031	50	26	30	3.13
23	Diazepam-d5	290.0	154.1	-	0.031	56	28	-	3.11
24	Diphenhydramine	256.2	167.1	152.0	0.024	22	16	50	2.24
25	Diphenhydramine -d3	259.2	167.1	-	0.024	22	14	-	2.24
26	Fentanyl	337.2	105.1	188.2	0.024	48	38	22	2.18
27	Fentanyl-d5	342.2	105.1	-	0.024	86	48	-	2.17
28	Hydrocodone	300.1	199.1	171.0	0.006	60	30	44	1.37
29	Hydrocodone-d3	303.2	199.1	-	0.006	94	34	-	1.37
30	Hydromorphone	286.1	185.1	157.1	0.008	66	32	42	1.00
31	Hydromorphone-d3	289.0	184.9	-	0.008	50	30	-	0.99
32	Ketamine	238.1	125.0	207.0	0.006	28	24	12	1.55
33	Ketamine-d4	242.1	129.0	-	0.006	62	26	-	1.55
34	MDA	180.2	163.2	133.1	0.006	18	8	16	1.33
35	MDA-d5	185.1	110.1	-	0.006	36	22	-	1.33
36	MDMA	194.1	163.1	105.0	0.006	24	11	24	1.40
37	MDMA-d5	199.1	165.1	-	0.006	46	14	-	1.40
38	Methadone	310.2	265.2	105.0	0.024	34	16	28	2.61
39	Methadone-d3	313.2	105.0	-	0.024	64	26	-	2.61
40	Methamphetamine	150.1	91.0	119.0	0.006	25	16	12	1.39
41	Methamphetamine-d5	155.1	91.9	-	0.006	52	18	-	1.39
42	Methaqualone	251.1	91.1	132.0	0.024	98	40	26	2.76
43	Methaqualone-d7	258.2	98.0	-	0.024	52	44	-	2.75
44	Methcathinone	164.0	131.0	105.0	0.006	24	18	26	1.20
45	Morphine	286.1	201.1	165.1	0.016	54	28	34	0.88
46	Morphine-d3	288.9	200.9	-	0.016	50	26	-	0.88
47	O-DSMT	250.1	58.0	-	0.006	54	16	-	1.35
48	O-DSMT-d6	256.2	64.1	-	0.006	54	16	-	1.35
49	Oxycodone	316.1	256.2	241.1	0.006	44	26	26	1.30
50	Oxycodone-d3	319.2	244.0	-	0.006	40	32	-	1.30
51	Oxymorphone	302.1	227.0	242.1	0.011	44	28	24	0.93
52	Oxymorphone-d3	305.1	230.0	-	0.011	36	30	-	0.93
53	THC-COOH	345.1	327.3	299.3	0.063	40	20	25	4.09
54	THC-COOH-d3	348.2	196.1	-	0.063	42	32	-	4.09
55	Tramadol	264.2	58.0	-	0.038	24	16	-	1.71
56	Tramadol-13C-d3	268.2	58.0	-	0.038	32	18	-	1.71

2.4 Data collection and management

Paired mortuary admission femoral blood and autopsy femoral and cardiac blood drug concentrations together with relevant case information were collated in Microsoft 365® Excel® (Microsoft, USA). Time points that were recorded include date and time of death declaration, date and time of mortuary admission, date and time of admission blood sample collection (F1), date and time of post-mortem and collection times for F2 and C samples. Case demographic information recorded included reported age (in years), sex and suspected cause of death.

As per the researcher's Data Management Plan, all information collected was collated by the researcher in a password protected and access-controlled Microsoft Excel database. The biological sample information was recorded in a secure sample repository database as per laboratory standard operating procedures by the

Forensic Toxicology Unit. After analysis, the instrumental raw data and processed data was stored in the project's OneDrive account accessible only by the researcher and supervisors.

2.5 Statistical analysis

All data was analysed using the statistical Package for Social Sciences (SPSS) Ver 28 (IBM Corp. Armonk, NY) Frequency statistics were generated for all categorical variables. Normality of numerical data was assessed using Shapiro Wilk test. The mean age between sexes was assessed using a student's t-test. Drug concentrations were not normally distributed, therefore differences between collection times or sites were assessed using a Friedmans test. Pairwise differences were assessed post-hoc using the Wilcoxon sign rank test with Bonferroni correction applied. Correlation between sample point ratios and PMI were assessed using Spearman's rank correlation. The level of significance was set at $\alpha = 0.05$ for all statistical tests.

2.6 Ethics

This research study was approved by the University of Cape Town's Human Research Ethics Committee (HREC 298/2022) (Appendix 2). Retrospective informed consent for sample collection was obtained by the researcher from the next-of-kin at the time of the identification process at the mortuary facility. Once the family arrived at the facility, the identification team informed the researcher, who would explain to the next-of-kin the need for the research and how the study was being conducted. In cases where the family did not speak English or Afrikaans, an interpreter was used. If the next-of-kin was willing to give consent, written consent was obtained on a standardised consent form (Appendix 3). The family was provided with a study information leaflet to take home, containing a description of the study and the researchers' contact details if the next-of-kin were to retract their consent or have further questions or queries regarding the study (Appendix 3). Only samples from cases where consent was obtained were included for analysis in the study.

3. Results

3.1 Case characteristics

Admission blood specimens were collected in 200 cases and consent for testing was obtained in 117 (58.5%) of these cases (Figure 2). In six cases, consent was obtained, however, autopsy specimens were not collected, or a full autopsy was not performed. In total, specimens from 109 (54.5%) cases with consent were analysed. Of these, 107 (98.1%) cases had all three specimens (F1, F2 and C) present, while in two cases only admission (F1) and autopsy (F2) femoral blood specimens were available. Drugs present on the panel were detected and quantified in one or more of the specimens in 61 cases (55.9%) (Figure 2 and Appendix 4). Drugs on the panel

were not detected in 48 (44.0%) cases, and two (1.8%) cases were not analysed at the time of study conclusion, thus these cases were excluded from further analysis. Of all positive cases, the most common analytes detected were acetaminophen (n=13; 21.3%), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) (n=20; 37.8%), amphetamine (n=30; 49.2%), methaqualone (n=33; 54.1%) and methamphetamine (n=33; 54.1%).

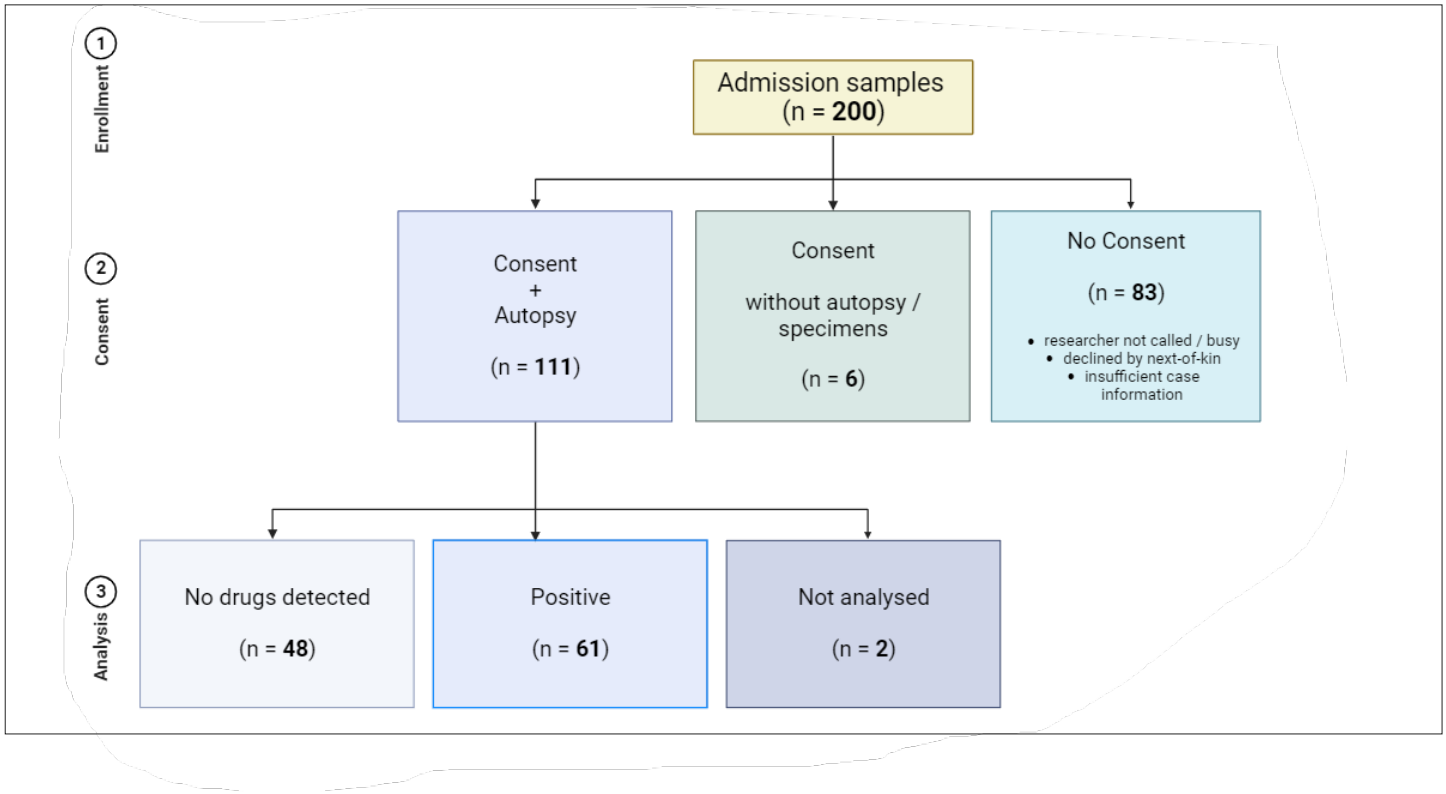

Figure 2: Overview of number of admission samples collected, attrition due to lack of full post-mortem or consent, the number of cases tested, and the number of cases yielding positive or negative results.

Table 2 describes the basic demographics and circumstances of admission of the cases tested (n=109). It should be noted that these may not represent the demographics of all decedents admitted to the mortuary during the study period. In this cohort, the mean age of individuals testing positive was 33 years (SD: 10 years), with no significant difference in mean age between males and females (p=0.2964).

Table 4. Overview of the number of cases analysed (n=109) and whether positive analytical results were obtained according to sex and the admission circumstances and/or cause of death.

Cause or circumstance of death	Analytes Detected [n (% of total cases)]		No Analytes Detected [n (% of total cases)]		Total Cases [n (%)]
	Male	Female	Male	Female	
Assault	3 (2.8)	-	1 (0.9)	-	4 (3.7)
Drown	-	-	1 (0.9)	-	1 (0.9)
Hang	6 (5.5)	2 (1.8)	6 (5.5)	1 (0.9)	15 (13.8)
Overdose	1 (0.9)	-	-	-	1 (0.9)
RTA[a] Passenger	2 (1.8)	-	-	-	2 (1.8)
RTA[a] Pedestrian	-	1 (0.9)	2 (1.8)	-	3 (2.8)
Shot	23 (21.1)	1 (0.9)	18 (16.5)	2 (1.8)	44 (40.4)
Stab	7 (6.4)	-	6 (5.5)	-	13 (11.9)
SUDA[b]	10 (9.2)	5 (4.6)	8 (7.3)	2 (1.8)	25 (22.9)

Unknown	-	-	1 (0.9)	-	1 (0.9)
Total [n (%)]	**52 (47.7)**	**9 (8.3)**	**43 (39.4)**	**5 (4.6)**	**109 (100)**

[a] RTA: Road traffic accident

[b] SUDA: Sudden unexpected death of an adult (this acronym is used at mortuary admission of a case where no clear information is available regarding a possible cause of death. These cases can therefore entail natural and unnatural causes of death. If a cause of death is identified. death after the autopsy and ancillary investigations, the case is no longer classified as a SUDA.

3.2 Admission and specimen collection times

Time points were recorded between the death declaration, admission to the mortuary, and collection of admission and autopsy specimens, and analysed for all included positive cases (n=61). The average post-mortem interval (PMI) (time between death declaration and autopsy) for positive cases was 81.1 hours (SD±44 hours), and the average time between admission sample collection and autopsy sample collection for positive cases was 76.8 hours and 79.7 hours for femoral and cardiac blood respectively (Table 3), compared to the provincial average of 75.12 hours from admission to autopsy.

Table 5. Number of cases with available data for each time point, together with the average and standard deviation times (hours) between those time points

Timepoints	Timepoint Summary	Number of cases with available data [n (% of total positive cases)]	Average Time ± Standard Deviation (hours)
Death Declaration to Mortuary Admission	Death ⟶ Admission	61 (100)	3.4 ± 1.7
Death Declaration to Admission Femoral Blood Collection (F1)	Death ⟶ F1	61 (100)	4.3 ± 3.3
Death Declaration to Autopsy Femoral Blood Collection (F2)	Death ⟶ F2	57 (93.4)	81.1 ± 44.0
Death Declaration to Autopsy Cardiac Blood Collection (C)	Death ⟶ C	55 (90.2)	81.7 ± 44.5
Mortuary Admission to Admission Femoral Blood Collection (F1)	Admission ⟶ F1	61 (100)	0.5 ± 0.6
Mortuary Admission to Autopsy Femoral Blood Collection (F2)	Admission ⟶ F2	57 (93.4)	78.7 + 45.1
Mortuary Admission to Autopsy Cardiac Blood Collection (C)	Admission ⟶ C	55 (90.2)	79.3 ± 45.6
Admission (F1) to Autopsy (F2) Femoral Blood Collection	F1 ⟶ F2	57 (93.4)	76.8 ± 43.9
Admission Femoral Blood (F1) to Autopsy Cardiac Blood Collection (C)	F1 ⟶ C	55 (90.2)	79.7 ± 45.1

Abbreviations: F1: Admission femoral blood; F2: Autopsy femoral blood; C: Autopsy cardiac blood.

3.3 Concentration Changes Between Post-mortem Specimens

Table 4 illustrates the number of cases for which analytes were positive, together with the mean concentration of those analytes within the admission femoral (F1), autopsy femoral (F2) and cardiac specimens. Where analytes were positive in five or more cases, these were assessed for statistical differences (Riffenburgh, 2012). Cases where concentrations were reported as below the limits of quantitation (e.g., <0.01 mg/L) were treated as 'missing' for concentration calculations but were included in total counts of positive cases.

Table 6. Number of cases positive for analytes for cases, with their mean femoral admission blood (F1), autopsy femoral blood (F2), and cardiac blood concentrations in mg/L (where above the limit of quantitation).

Analyte	Number of cases positive for analyte [n (% of total)]	Admission Femoral Blood (F1) Conc. [Mean (SD) (mg/L)]	Autopsy Femoral Blood (F2) Conc. [Mean (SD) (mg/L)]	Cardiac Blood (C) Conc. [Mean (SD) (mg/L)]	*p*-value
6-Acetylmorphine	1 (0.9)	-	0.01	-	-
Acetaminophen	13 (11.9)	7.3 (12.9)	5.95 (11.94)	8.43 (14.98)	0.07
Alprazolam	2 (1.8)	0.01	-	0.01	-
Amphetamine	30 (27.5)	**0.05 (0.05)[a]**	**0.07 (0.04)[b]**	**0.17 (0.14)[ab]**	**<0.001**
Benzoylecgonine	2 (1.8)	0.14 (0.17)	0.1 (0.09)	0.13 (0.15)	-
Clobazam	1 (0.9)	0.10	0.15	0.19	-
Cocaine	1 (0.9)	0.02	0.02	0.02	-
Codeine	1 (0.9)	0.15	0.2	0.34	-
Diazepam	6 (5.5)	0.07 (0.06)	0.05 (0.03)	0.06 (0.02)	0.223
Diphenhydramine	11 (10.1)	0.1 (0.1)	0.09 (0.08)	0.15 (0.15)	0.097
MDA *	1 (0.9)	0.01	0.02	0.06	-
MDMA **	1 (0.9)	0.49	0.44	4.06	-
Methadone	1 (0.9)	0.13	0.08	0.14	-
Methamphetamine	33 (30.3)	**0.36 (0.34)[a]**	**0.51 (0.51)[a]**	**1.08 (1.2)[a]**	**<0.001**
Methaqualone	33 (30.3)	1.08 (0.70)	1.06 (0.67)	1.23 (0.78)	0.131
Morphine	3 (2.8)	0.04	0.03 (0.02)	0.25 (0.31)	-
O-desmethyltramadol	2 (1.8)	0.01	0.01	0.01	-
THC-COOH***	20 (18.4)	**0.05 (0.04)[a]**	**0.04 (0.06)**	**0.04 (0.03)[a]**	**0.017**
Tramadol	3 (2.8)	0.07	0.06 (0.04)	0.06 (0.04)	-

[a,b] Denotes significant pairwise differences with Bonferroni correction
* Methylenedioxyamphetamine
** 3,4-Methylenedioxymethamphetamine
*** 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol

Significant pairwise differences were found between both admission (F1) and cardiac (C) as well as autopsy (F2) and cardiac (C) blood concentrations for amphetamine. Methamphetamine demonstrated significant differences between all specimens (F1, F2, and C). Significant differences were also observed for THC-COOH admission (F1) and cardiac (C) blood specimens. No significant changes in concentrations between specimens were observed for diazepam (n=6), diphenhydramine (n=11), and methaqualone (n=33).

3.4 Correlation of F2/F1 and C/F2 with Post-mortem Interval (PMI)

The mean autopsy/admission (F2/F1) and cardiac/autopsy (C/F2) ratios for analytes positive in five or more cases are presented in Table 5. Diazepam was removed from correlation analysis as only two samples with values for both measures were available. F2/F1 ratios were correlated against the time between the two samples. C/F2 was correlated against the time since death. No C/F2 ratios were significantly correlated with time. The F2/F1 ratio for amphetamine had a moderate association with time between the collection of the two specimens.

Table 7. Mean ratios of F2/F1 and C/F2 correlated with time between specimen collection and PMI, respectively.

Analyte	Autopsy/Admission Femoral (F2/F1) Ratio			Cardiac/Autopsy Femoral (C/F2) Ratio		
	Mean Ratio (SD)	r_s	*p*-value	Mean Ratio (SD)	r_s	*p*-value
Acetaminophen	1.55 (0.72)	0.0455	0.8944	1.31 (0.62)	0.2909	0.3855
Amphetamine	1.71 (1.04)	0.4455	**0.0377**	2.61 (1.75)	-0.1354	0.538
Diphenhydramine	1.63 (1.28)	-0.5	0.6667	1.59 (0.52)	0.7	0.1881
Methamphetamine	1.81 (1.67)	0.3095	0.0961	2.28 (1.44)	0.1005	0.604
Methaqualone	0.99 (0.27)	0.2494	0.1838	1.21 (0.49)	-0.0345	0.8617
THC-COOH*	0.84 (0.65)	-0.0971	0.7207	1.33 (1.17)	-0.0321	0.9095

*11-nor-9-carboxy-Δ^9-tetrahydrocannabinol

The mean autopsy/admission (F2/F1) and cardiac/autopsy (C/F2) ratios are further represented graphically in Figure 3.

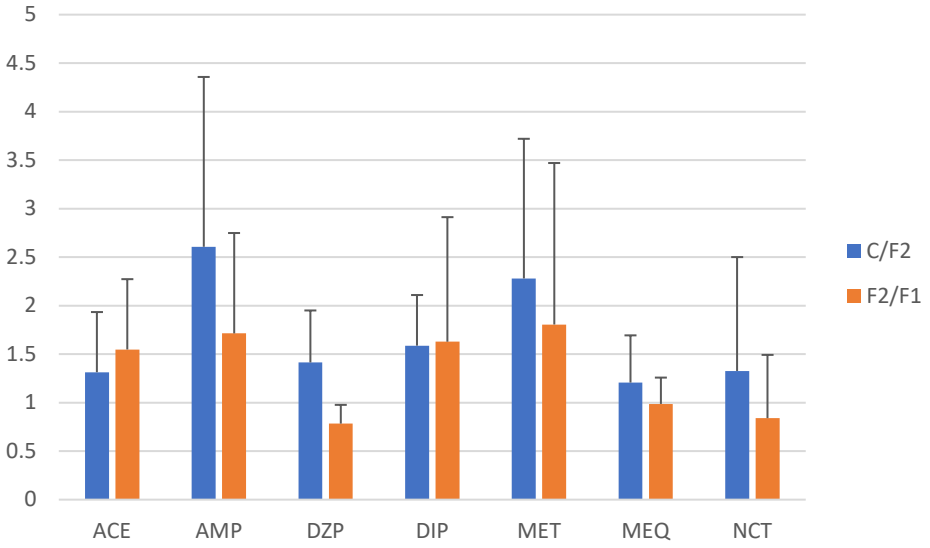

Figure 3: Mean sample ratio concentration ratios of autopsy/admission femoral (F2/F1) blood concentrations, and cardiac/autopsy femoral (C/F2) concentrations for positive analytes with more than five detections. Error bars indicate +1 SD. (Abbreviations: ACE: acetaminophen; AMP: amphetamine; DZP: diazepam; DIP: diphenhydramine; MET: methamphetamine; MEQ: methaqualone; NCT: THC-COOH)

4. Discussion

This pilot study investigated post-mortem redistribution between admission and autopsy specimens for commonly misused drugs in Cape Town, South Africa, for which sufficient positive cases for seven drugs permitted further statistical assessment. In this study PMR was evaluated using C/F2 (or cardiac/peripheral autopsy concentrations) and F2/F1 (autopsy/admission concentrations) concentration ratios as proxies.

In this study, most cases included in the cohort were males. Males are overrepresented in the mortuary population in the Western Cape (Evans et al., 2018). While suspected cause of death was recorded, further analyses to evaluate the relationship of the drug concentrations and the cause of death were not performed as these cases represented a minor cohort of cases in that time frame, highlighting the need for further studies to evaluate this relationship.

The average pre-autopsy interval in the current study, of 81.1 ± 44.0 hours, was slightly longer than those of previous studies, which had average intervals of 64 hours and 65.2 hours (Gerostamoulos et al., 2012; Kamphuis et al., 2021). This may be a consequence of the high case load seen at Salt River Mortuary. Despite this longer PMI, there was no significant relationship between PMR and the pre-autopsy interval for any of the drugs, which is in agreement with a previous study (Gerostamoulos et al., 2012).

Significant pairwise differences in concentrations between specimen types were observed for amphetamine (between peripheral and cardiac blood), methamphetamine (all specimens) and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (between admission femoral and autopsy cardiac blood). Concentrations in cardiac blood were significantly higher than autopsy and/or admission femoral blood for amphetamine and methamphetamine, the latter is in agreement with previous literature (McIntyre, I., 2011). This highlights the possible pitfall for overinterpreting findings if only cardiac blood were to be analysed. Conversely, overinterpretation of femoral concentrations in isolation may also occur, as McIntyre revealed a 1.5-fold concentration increase from antemortem to post-mortem samples (McIntyre, I., 2011). In the local context, cardiac blood may be the only specimen available in infant and young child deaths, burn and trauma cases, which are all frequently encountered. In contrast, Gerostamoulos, et al. (2012) reported statistically insignificant femoral amphetamine and methamphetamine concentration decreases from admission to autopsy, despite these drugs having the largest concentration changes (Gerostamoulos et al., 2012). The latter once again highlighting the inconsistent concentration changes and myriad of factors which may influence PMR.

Amphetamine and nor-carboxy-tetrahydrocannabinol had no significant difference when comparing admission femoral (F1) and autopsy femoral (F2) concentrations, supporting the recommendations for choosing peripheral blood samples over central sources when collecting specimens for toxicological analyses at the time of autopsy, as this is likely to be a more accurate representation of the concentrations at the time of death. There was also a significant difference in concentration when comparing results from autopsy femoral blood (F2) and autopsy cardiac blood (C), even though these are usually collected within a short timespan from one another, once again emphasising that central blood samples tend to reveal higher drug concentrations. The latter may be as a result of diffusion from nearby drug reservoirs, such as the stomach and liver and/or concentration of drugs within the heart tissue. It is interesting to note that methaqualone (n =33) revealed no significant concentration differences between the different time points, suggesting that the drug may not undergo significant redistribution in the post-mortem period. No data is available for PMR of Methaqualone. This drug is rapidly absorbed from the digestive tract and concentrated in adipose and brain tissue after administration and released over several days (Nogué et al., 1996). This may suggest it is prone to redistribution, however, this was not observed in this sub-set of data and warrants further investigation.

A study comparing antemortem methamphetamine and amphetamine concentrations to those found in peripheral post-mortem blood revealed that post-mortem blood concentrations may be up to 1.5-fold more than ante-mortem concentrations (McIntyre, I. M. et al., 2013). The authors additionally highlighted that the effect of PMR in peripheral blood should not be disregarded. Additionally, the study highlighted a smaller concentration change with a decreased PMI (McIntyre, I. M. et al., 2013). These may be the reasons for methamphetamine showing significant differences between admission and autopsy femoral concentrations in the current study. Given the significance of methamphetamine being one of the most frequent drugs misused in Cape Town, besides minimising PMI (which didn't show significant changes), it may be recommended to collect admission specimens in cases where a methamphetamine overdose is suspected. Amphetamine (n = 13; 11.9 %) was the only drug in this study which showed significant concentration differences between F1 and F2 when PMI specifically was considered (Table 5), supporting the recommendations that samples must be collected as soon as possible after death.

C/P ratios for acetaminophen, amphetamine, methamphetamine and nor-carboxy-tetrahydrocannabinol in the current study were similar to previous studies (Table 6) falling within the ranges reported in these studies. All ratios were >1 suggesting, even with the small sample size, to support the indication that drug concentrations in cardiac blood are typically higher than femoral. C/P ratios for acetaminophen, amphetamine, diphenhydramine, methamphetamine, methaqualone and nor-carboxy-tetrahydrocannabinol were above 1, suggesting that they undergo PMR to some extent. Acetaminophen, methaqualone and nor-carboxy-tetrahydrocannabinol had C/P ratios were close to one, which may indicate that these drugs undergo PMR to a lesser extent than the other drugs.

Table 8*:* Comparison of C/P ratios between the current study and selected publications

Analyte	Mean C/P ratio in current study (number of positive cases)	Mean C/P ratio (n)	Median C/P ratio	C/P ratio range	Literature reference
Acetaminophen	**1.3 (13)**	1.3 (37)	-	0.7 – 2.8	(M. Dalpe-Scott, 1995)
		1.5 (4)	-	1.1 – 2.5	(Yonemitsu & Pounder, 1992)
Amphetamine	**2.6 (30)**	2.0 (1)	-	-	(M. Dalpe-Scott, 1995)
		2.4 (20)	-	1.2 – 5.6	(Barnhart, Fogacci & Reed, 1999)
		2.1 (75)	1.9	0.67 – 6.6	(de Groot et al., 2023)
Diphenhydramine	**1.6 (11)**	2.3 (32)	-	0.8 - 21	(M. Dalpe-Scott, 1995)
		2.4 (7)	-	0.4 – 6.0	(Anderson & Prouty, 1989; Roettger, 1990)
Methamphetamine	**2.3 (33)**	1.6 (18)	-	0.9 -2.4	(McIntyre, I., 2011)
		2.4 (1)	-	-	(M. Dalpe-Scott, 1995)
		1.9 (5)	-	1.0 – 3.8	(Prouty & Anderson, 1990)
		2.1 (20)	-	1.2 – 5.0	(Barnhart, Fogacci & Reed, 1999)
Methaqualone	**1.2 (33)**	-	-	-	-

THC-COOH	**1.3 (20)**	-	1.3	-	(Hoffman et al., 2020)
		1.5 (17)*	1.2	0.3 – 3.1	(Meneses & Hernandez, 2021)
		1.4 (16)**	1.3	0.3 – 2.4	
		1.5	1.4	0.35 – 6.2	(Tascon et al., 2023)

*FID: Fatally injured driver

**non-FID: non-fatally injured driver cases (individuals who were not driving a vehicle at the time of death)

The analytical method utilised did not analyse for tetrahydrocannabinol due to poor validation results, thus only THC-COOH was assessed in this study. While a mean C/F2 > 1 was obtained, the opposite was observed for autopsy/admission (F2/F1) ratios, however, the overall mean concentrations of these three groups were very similar. A recent study by Tascon et al. (2023) provides the most up-to-date assessment of PMR of Δ^9-THC (THC) and its metabolites, finding that THC-COOH redistributed toward central blood (median C/P ratio of 1.3). This was also demonstrated in this study, as was the finding that there were no statistical correlations to PMI. Tascon et al. (2023) indicated that they could not correlate changes in PMR to body-mass index (BMI), state of decomposition, or PMI. The authors further suggested that mechanisms for this distribution may include enterohepatic recirculation of THC-COOH-glucuronide and cleavage to THC-COOH in the small intestine, as well as laboratory workflows in terms of order of analyses and storage conditions of specimens, which may alter concentrations due to *in vitro* degradation of THC-COOH-glucuronide to THC-COOH (Tascon et al., 2023).

Key recommendations made by Tascon et al. (2023) included the analysis of cardiac blood, peripheral (femoral) blood, and urine in all cases in a close time frame (for comparison). While this was specific to Δ^9-THC and its metabolites, it could be applied to other drugs as well in order to routinely assess the role of PMR in casework. They also recommend the reporting of these cannabinoids as only detected or not detected, and it is recommended that a similar approach be taken locally, and especially for THC-COOH (an inactive metabolite) given the complexities associated with interpreting concentrations.

4.1 Study Strengths

In this research study, paired results from three specimen types were assessed: F1/F2, F1/C, and F2/C. Specimens from a single case were analysed in one batch, a method that helps minimise any alterations to the comparative concentrations among these specimen types, although it should be noted that there may have been slight changes in actual concentration over time depending on time frame of analysis. It is important to highlight that F1 provides a closer representation of concentrations at the time of death, thereby enabling a more reliable determination of its contribution to the cause of death. The added advantage of using the newly established inhouse laboratory to do the analysis, removes the historical problem of long waiting periods between collection and processing of samples at the National Health laboratories that were previously utilised for these purposes, and the resultant issues with interpretation.

This study distinguishes itself by offering novel insights into drugs that have received limited international attention, thereby contributing original knowledge to the field. By focusing on less commonly studied substances, it underscores the importance of broadening the scope of research within forensic toxicology. The dissertation's findings hold significant implications for forensic practices, particularly in regions where these drugs are more prevalent. By shedding light on their pharmacological properties and effects, the study can inform forensic toxicologists about the unique challenges associated with these substances, potentially leading to adjustments in forensic protocols. Moreover, this research advocates for a more globally inclusive approach to studying drugs, highlighting the need for increased attention to regionally significant substances. By advocating for greater international collaboration and research efforts, this study aims to expand the global knowledge base in forensic toxicology, ultimately enhancing our understanding of diverse substances encountered in forensic contexts worldwide.

4.2 Study Limitations

In the context of this research study, several limitations warrant consideration. Firstly, it is essential to acknowledge that the study was performed in a busy mortuary where ideal experimental scenarios are replaced by what is practically feasible and attainable with the available staff and resources. This was particularly evident in the inconsistent communication from mortuary staff regarding admissions and familial consent. This does, however, demonstrate the results that would be obtained in routine settings. Furthermore, it is important to note that the declaration of death may not consistently align with the actual time of death but rather when the deceased individuals were discovered and officially declared deceased. Additionally, significant variability exists in the durations between the declaration of death and the subsequent admission to the mortuary, introducing potential confounding factors. The latter may be due to lack of mortuary vehicles to fetch bodies or a single vehicle having to travel to various scenes for body collection prior to returning to the mortuary.

The study is further constrained by the relatively small number of cases available for analysis. Moreover, drug testing was limited to the examination of the 31 most commonly encountered substances. The methodology employed for admission sample collection relied on a blind-stick approach, and ethics permission was not granted for blind stick F2 collection, potentially excluding pertinent cases if full autopsy was not performed. Furthermore, religious considerations also played a role, as some religions and cultures prohibit the removal of bodily substances for research, limiting the ability to obtain consent. The generalisability of the findings to other drugs is restricted, as each drug or medication necessitates independent investigation. Importantly, the study did not assess the effects of decomposition, body-mass index, resuscitation, or underlying medical conditions on the parameters of interest. The latter is due to the scanty information available to the pathologist prior to the post-mortem, as the next-of-kin or by-standers at the scene of death either do not know or understand the decedents' medical history or are reluctant to divulge such information. The limited

number of cases available posed a challenge when attempting to compare results between trauma and non-trauma cases. Furthermore, it should be noted that factors such as changes in haematocrit, protein binding, and pH due to post-mortem changes and body position could not be adequately controlled. Finally, some collection times were not consistently documented or not documented at all, potentially affecting the accuracy and completeness of the dataset. These limitations should be taken into account when interpreting the study's findings.

4.3 Recommendations

To strengthen and build on the current study, future studies should consider including larger case numbers and expanding the panel of drugs which are tested for. This would enable contributing more data to the current pool of information. Additionally, evaluating concentration ratios between whole blood and vitreous humour can prove helpful, as vitreous is less susceptible to PMR processes (Pigaiani et al., 2020). Adding more specimen types, such as urine and bile can add additional knowledge on PMR. A cut-down method of sampling in conjunction with vessel ligation may improve reliability of femoral results. In addition, having a single pathologist perform all blood collections, would permit consistency in experimental procedures, but may not necessarily represent routine conditions. To this end, it is recommended that universal (at Salt River Mortuary and throughout the province) sampling techniques be implemented, as well as training the Forensic Officers to recognise potential toxicological cases, to collect admission blood samples and flagging the cases for prioritisation of autopsy, thereby decreasing the PMI. The latter may prove difficult considering the mortuary's resource constraints and high case load.

5. Conclusion

To the best of the authors' knowledge, this is the first study on PMR in South Africa. The results of 109 authentic autopsy cases in a practical mortuary setting were analysed for the 31 most commonly abused drugs in the mortuary's drainage area. This included cases with short and long post-mortem intervals. This study adds valuable data to the existing research with regards to the propensity, extent and potential of post-mortem redistribution of acetaminophen, nor-carboxy-tetrahydrocannabinol, amphetamine, methaqualone and methamphetamine. Methamphetamine was noted to undergo significant changes between admission and autopsy samples (both femoral and cardiac), and care must be taken in interpreting concentrations. Methaqualone, however, showed limited propensity towards PMR, however, this should be confirmed with further studies. The study further provides insight into the burden of drug misuse in the setting of deaths in the West Metropole of Cape Town. While there is currently no single solution to eliminate all the factors causing these difficulties, there are strategies to overcome some of the problems when facing PMR. A multipronged and individualised approach should be followed throughout the entire post-mortem process for

each case. Starting at minimising the PMI, continuing through to appropriate sampling technique and storage, sample preparation and analysis, result reporting and possible alternative strategies.

CRediT authorship contribution statement:

Gavin Martin Kirk: Writing – Review & Editing, Supervision, **Bronwen Beth Davies:** Conceptualisation, Methodology, Validation, Data Review, Resources, Writing – Review & Editing, Supervision, Funding acquisition, **Marie Belle Kathrina Mendoza Hlela:** Methodology, Validation, Toxicological analyses and data review, Supervision, **Liza Clegg:** Methodology, Investigation, Data Curation, Writing – Original and Final Draft, Visualization, Project Administration

Declarations of interest: None

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Appendices

1. Instructions to authors from Forensic Science International

FORENSIC SCIENCE INTERNATIONAL

An international journal dedicated to the applications of medicine and science in the administration of justice.

AUTHOR INFORMATION PACK

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DESCRIPTION

Forensic Science International is the flagship journal in the prestigious Forensic Science International family, publishing the most innovative, cutting-edge, and influential contributions across the forensic sciences. Fields include: forensic pathology and histochemistry, chemistry, biochemistry and toxicology, biology, serology, odontology, psychiatry, anthropology, digital forensics, the physical sciences, firearms, and document examination, as well as investigations of value to public health in its broadest sense, and the important marginal area where science and medicine interact with the law.

The journal publishes:Case Reports Commentaries Letters to the Editor Original Research Papers (Regular Papers) Rapid Communications Review Articles Technical Notes

Forensic Science International adheres to strict ethical publication guidelines and actively supports a culture of inclusive and representative publication. For any submission enquiries, please contact the respective Editor.

The Forensic Science International journals offer comprehensive and pioneering coverage within the forensic sciences and beyond, disseminating ground-breaking discoveries, highly specialised research, and foundational science across the family of publications. The FSI portfolio comprises of: Forensic Science InternationalForensic Science International: Animals and EnvironmentsForensic Science International: Digital InvestigationForensic Science International: Genetics Forensic Science International: Genetics Supplement SeriesForensic Science International: Mind and LawForensic Science International: ReportsForensic Science International: Synergy

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Pathologists, Anthropologists, Psychiatrists, Biologists, Serologists, Odontologists, Physical Scientists, Toxicologists, Scientists in Legal and Social Medicine, Questioned Documents and Jurisprudence

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2. Ethics approval letter from the Faculty of Research Ethics Committee

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

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

17 October 2022

HREC REF: 298/2022

Dr G Kirk
Department of Forensic Medicine & Toxicology
Falmouth Building -FHS
Email: gavin.kirk@uct.ac.za
Student: liza.clegg@uct.ac.za

Dear Dr Kirk

PROJECT TITLE: INVESTIGATING POST-MORTEM REDISTRIBUTION OF DRUGS IN A COHORT OF UNNATURAL DEATHS IN CAPE TOWN SOUTH AFRICA-
(MMED CANDIDATE-DR LIZA CLEGG)

Thank you for your response letter, addressing the issues raised by the Faculty of Health Sciences 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 October 2023.

Please submit a progress form, using the standardised Annual Report Form (FHS016) if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Dr Liza Clegg will also be involved in this study.

Please quote the HREC REF 298/2022 in all your correspondence.

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

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

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938 NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2020), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC/ref 298.2022

3. Informed consent information sheet and form: English

INFORMATION SHEET FOR PARTICIPANTS' NEXT-OF-KIN

Research Project:
Investigating post-mortem redistribution (PMR) of drugs in a cohort of unnatural deaths in Cape Town South Africa

---

Supervisor: Dr G. Kirk
Co-supervisors: B. Davies and K. Hlela
Researcher: Dr L. Clegg

Who are we?
You are invited to participate in a collaborative study with the Division of Forensic Medicine and Toxicology at the University of Cape Town (UCT) and Salt River mortuary. It will be conducted by forensic pathologists Dr. Liza Clegg and Dr. Gavin Kirk, and toxicologists Bronwen Davies and Kathrina Hlela.

The purpose of the research study is to try and understand how the amount of drug changes in blood after a person dies. This form explains what you will be asked to do if you decide to participate in this study. Please read it carefully and feel free to ask any questions you like before you decide about participating.

Background information:
When an individual passes away and it is suspected that they may have died unnaturally, an autopsy must be conducted by an authorised forensic doctor at a Western Cape, Department of Health and Wellness, Forensic Pathology Service (FPS) mortuary. As part of the routine autopsy procedure, the doctor will collect blood for further testing to assist in determining the cause of the person's death. One of the important investigations performed is a toxicological analysis to determine whether the individual was exposed to any drugs or toxic substances. This exposure may have been on purpose or completely by accident. Testing for the presence of drugs or toxic substances is performed in most autopsies as routine services. If substances are found, it can help the doctor in determining the cause of death.

However, the amount of drug in blood may change after a person dies. Often the autopsy takes place a few days after the individual is admitted to the mortuary. This can complicate the interpretation of the cause of death. If a blood specimen is taken at the time of admission to the mortuary (before autopsy), the amount of substances in blood is similar to that around the time of death. This may give a better picture of the role of these substances in death.

What is the purpose of this study?
In this study, we aim to describe the changes in the amount of drug(s) in blood, between admission to the mortuary and at autopsy (taken at different sites - from the leg and from the center of the body). This information will eventually be able to help improve procedures in testing at the mortuary for future death cases.

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 has passed away in an unnatural manner. In order for us to do this study, we need to ask you if we may collect three (3) blood samples from your next-of-kin.

Procedure

Blood (approximately 3 mL) will be collected by an authorised Forensic Pathologist from a blood vessel in the leg when the deceased is admitted to Salt River mortuary. Additional blood samples from the heart and the blood vessel in the leg will be collected at the time of the autopsy during routine collection procedures (also 3 mLs each). The collection of these samples will not affect the normal autopsy procedures and will not pose any additional harm to your next-of-kin's body.

These blood samples will be tested for the common drugs identified in Cape Town by the Forensic Toxicology Unit laboratory in FPS/UCT.

The name of your family member will not be made known. Instead, the samples and the toxicological data produced will be anonymized, which means that any identifiers will be replaced with numbers or symbols. The confidentiality of the samples and data will be maintained in the following ways:

1. Every person involved in the project completes a confidentiality agreement as part of the FTU quality management system.
2. Only the primary investigators will have access to the database.
3. All samples, data and results generated will be stored in password protected spreadsheets.
4. The results of the study may be published or presented at meetings but the identity of the deceased and the family will not be revealed.
5. Your participation will be kept confidential.

What happens if you attend the mortuary after the autopsy has been performed?

The autopsy and collection of samples usually happen after obtaining consent and identifying the body. However, sometimes the autopsy is scheduled before the body identification is done and thus, the interview for obtaining your consent is done after the samples have already been collected. In such instances, the samples will be collected and stored until delayed consent is obtained from the decedent's next-of-kin / relative / family. In situations where delayed consent cannot be obtained or consent is declined, the respective samples will be destroyed. This will not affect the outcome of the autopsy procedure, or the services delivered by FPS (Forensic Pathology Services). Your consent is completely voluntary, and you may refuse participation at any time during the course of the study.

Making your choice

Your contribution is completely voluntary and will not cost you anything. You are free to decline participation at any time, for whatever reason, without negative consequences. Should you be willing to give consent, you will be asked to sign a consent form. You may withdraw your consent from the study at any time, for any reason. If the sample has already been analysed at the time you change your mind, your results will be removed from the secured database. That means that no additional researchers can get the data.

Your decision on whether to give consent will not affect any services provided to you by the FPS (Forensic Pathology Services).

What happens to the results?

The results will be provided to the Forensic Pathologist who performed the autopsy as part of their routine post-mortem investigation. The results will not be provided to the next-of-kin through this research. The information gathered may be used by the researchers to publish in research journals and/or presented at meetings or conferences.

How will samples be stored and used?

The samples will be stored at the Forensic Toxicology Unit laboratory based at the Division of Forensic Medicine and Toxicology, UCT. Storage and handling will be done based on standard operating procedures for biological sample storage as part of their operational casework and non-casework processes.

You will be asked if you will allow that the samples collected be stored for a longer period of time over and above this study (e.g. 5 years). You will further be asked whether additional studies stemming from this study may be conducted on the samples. These studies would only pertain to post-mortem redistribution investigations. You are under *no* obligation to store these samples for longer than this study, nor are you required to participate in future studies. Not participating in future studies will in no way affect the outcome of the post-mortem or this research project.

Ethical approval:

The biological samples will not be used for any research unless the research study is 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 studies.

If you agree to take part in this study, please read the consent form clearly and sign. After reading each sentence; please tick the Yes or No box. No matter what you decide, it will not negatively affect you or your deceased family member in any way. If you may have any questions or require referral to a grief centre or psychological support please don't hesitate to ask the person taking the consent. If you have any further queries about the study, please contact:

Supervisor: Dr Gavin Kirk | Tel: 021 406 6192 | Email: gavin.kirk@uct.ac.za
Researcher: Dr. Liza Clegg | Tel: 021 406 6192 | Email: liza.clegg@uct.ac.za
Human Research Ethics Committee Chairperson, Prof Marc Blockman | Tel: 021 406 6492 | Email: hrec-enquiries@uct.ac.za

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 have been informed about the purpose of the study and the procedures.		
3. I understand that participation is voluntary		
4. I am aware that I may withdraw from the study at any time without reason or consequence whether before or during the study		
5. Anonymity will be maintained and neither the deceased nor my family will be identified		

I consent to:

	Yes	No
The collection of three blood samples from the deceased for toxicological analyses.		

I understand that:

	Yes	No
The treatment and management of the biological samples of my deceased family member will be in accordance with guidelines of the University of Cape Town Faculty Of Health Science Human Research Ethics Committee.		
The scientific laboratories are under obligation to respect medical confidentiality.		
I can at any time withdraw my consent and that I must notify the primary investigator of my decision to withdraw.		
Research conducted with these samples may result in a publication, but neither the deceased nor the family of the deceased will be identified.		

Please note that the following section is voluntary. If you select "No" to any of the below, it will not affect the outcome of the study nor will it have any effect on the normal autopsy process:

I consent to:

	Yes	No
The samples being stored at the University of Cape Town for a period of 5 years over and above this study after which they will be appropriately discarded.		
The stored blood samples being used in further research studies over the 5 years, which have been reviewed and approved by the University of Cape Town, Faculty of Health Sciences, Human Research Ethics Committee.		

Full name of person obtaining consent

Signature of person obtaining consenting Date

Full name of person authorising consent for collection of samples at autopsy for use in research study Date

Signature of witness Date

Thumb print of the spouse/partner/major child/parent/guardian/major brother/major sister of the deceased:

4. Informed consent information sheet and form: Afrikaans

INLIGTINGSTUK VIR DEELNEMER SE NAASBESTAANDES

Navorsingsprojek:
Die ondersoek van nadoodse (post-mortem) herdistribusie (PMH) van middels in 'n groep (kohort) van onnatuurlike sterftes te Kaapstad, Suid-Afrika.

Studieleier:	Dr G. Kirk
Hulp-studieleier:	B. Davies en K. Hlela
Navorser:	Dr L. Clegg

Wie is ons?
U word uitgenooi om deel te neem aan 'n samewerkende studie van die Afdeling van Forensiese Medisyne en Toksikologie aan die Universiteit van Kaapstad (UK) en Soutrivier Lykshuis (Salt River Mortuary). Dit sal deur patoloë Dr. Liza Clegg en Dr. Gavin Kirk, asook toksikoloë Bronwen Davies en Kathrina Hlela, uitgevoer word.

Die doel van hierdie navorsingstudie is om te prober uitvind hoe die hoeveelheid van 'n middel (soos medikasie of dwelms) in 'n mens se bloed na die dood verander. Hierdie vorm verduidelik wat van u gevra gaan word, indien u sou besluit om aan hierdie navorsingstudie deel te neem. Lees asseblief (asb.) aandagtig deur en voel vry om enige vrae te vra wat u mag hê, voordat u oor deelname aan die studie besluit.

Agtergrondsinligting:
Wanneer 'n mens in Kaapstad sterf en daar word vermoed dat die persoon van 'n onnatuurlike oorsaak gesterf het, moet 'n nadoodse ondersoek (deur 'n gemagtigde forensiese dokter van die Wes-Kaap se Departement van Gesondheid se Forensiese Patologie-dienste lykshuise) uitgevoer word.

As deel van die gemiddelde nadoodse ondersoek, mag die dokter bloed vat om verskillende toetse op te doen om te help bepaal wat die oorsaak van dood was.

Een van die belangrike toetse is 'n toksikologie analise, om te bepaal of die persoon aan enige dwelms of toksiese middels blootgestel was. Hierdie blootstelling mag doelbewus of deur blote toeval plaasgevind het. Om te toets vir die teenwoordigheid van middels of toksiese stowwe, word roetinegewys tydens die meeste nadoodse ondersoeke gedoen. Indien daar 'n middel in die bloed gekry word, kan dit die dokter help om die oorsaak van dood te bepaal.

Die hoeveelheid van 'n middel wat in 'n mens se bloed is, kan na die dood verander. Dikwels word die nadoodse ondersoek 'n paar dae nadat die liggaam by die lykshuis ontvang is, gedoen. Dit kan dus die interpretasie van die oorsaak van dood beïnvloed. Indien bloed tydens die ontvangs van die liggaam by die lykshuis (vóór die nadoodse ondersoek) getrek word, dan behoort die bloed-vlakke van die middel amper dieselfde te wees as wat die vlakke was op die tyd wat die persoon gesterf het. Dit kan dan moontlik 'n beter aanduiding wees van hoeveel die middel tot die persoon se dood bygedra het.

Wat is die doel van hierdie studie?
Met hierdie navorsingstudie, be-oog ons om te ondersoek hoe 'n middel se bloedvlakke verander [wat van twee verskillende plekke van die liggaam af geneem word ('n onderste ledemaat/been en sentraal van die liggaam)], vanaf die tyd wat die liggaam by die lykshuis ontvang word en die tyd wat die nadoodse ondersoek uitgevoer word, en te omskryf.
Hierdie inligting sal dan kan help om die proses van hierdie toetse by die lykshuise, vir toekomstige sterfte gevalle, te verbeter.

Hoekom word u ondervra?
U word gevra om in te stem tot 'n onderhoud omdat u familielid / naasbestaande op 'n onnatuurlike wyse gesterf het en by Soutriver Lykshuis is. Om ons te help om die studie uit te voer, moet ons u vra of ons drie (3) bloedmonsters (minder as 1 eetlepel se volume in totaal) van u famielid / naasbestaande mag neem vir die studie.

Prosedure
Bloed (ongeveer 3mL – minder as 'n teelepel) sal deur 'n gemagtidgde Forensiese Patoloog vanuit die bloedvate van die been van die oorledene, te Soutriver Lykshuis, geneem word.
'n Verdere 2 bloedmosters (ook ongeveer 3mL elk) sal tydens die nadoodse ondersoek vanuit die hart en die bloedvate van die been geneem word, as deel van die gewone roetine nadoodse ondersoeke.
Die gewone nadoodse ondersoek en prosedures sal geensins deur die neem van hierdie bloedmonsters beïnvloed of addisionele skade aan u naasbestaande se liggaam veroorsaak nie.
Hierdie bloedmonsters sal dan vir die algemene dwelms en middels in Kaapstad by die Forensiese Patologie Dienste se Forensiese Toksikologie Eenheid ("Forensic Toxicology Unit" of "FTU") laboratorium van die UK ("UCT") getoets word.

U naasbestaande se naam sal nie bekend gemaak word nie. Die bloedmonsters en die toksikologiese data prosedure sal anoniem uitgevoer word. Dit beteken dat enige identifiserende persoonlike inligting met nommers of simbole vervang word.

Konfidensialiteit van die bloedmonsters en data sal op die volgende maniere onderhou word:

1. Almal wat by hierdie projek betrokke is, sal 'n konfidentialiteits ooreenkoms (as deel van die Forensiese Toksikologie Eenheid se Kwaliteitbestuurstelsel) teken. Slegs die hoof navorsers sal toegang tot die databasis hê.
2. Die inligting (data) en resultate wat met hierdie studie verband hou, sal in wagwoordbeskermde sigblaaie ("spreadsheets") gestoor word.
3. Die resultate van die studie kan gepubliseer of tydens vergaderings aangebied word, maar die identiteit van die oorledene sal nie bekend gemaak word nie.
4. U deelname aan hierdie studie sal ook konfidensieel gehou word.

Wat gaan gebeur as u die lykshuis na die nadoodse ondersoek besoek?
Die insameling van die bloedmonsters en nadoodse ondersoek word gewoonlik gedoen nadat die liggaam deur die familie geïdentifiseer is.

Die nadoodse ondersoek word egter soms geboek en uitgevoer, voordat die liggaam deur die familie geïdentifiseer is. Daarom sal die onderhoud en toestemming nadat die bloedmonsters geneem is, gedoen kan word. In sulke gevalle sal die bloedmonsters geneem en gestoor word totdat toestemming vanaf naasbestaande of familie verkry is.

In gevalle waar toestemming nie verkry kan word nie of toestemming geweier word, sal die bloedmonsters vernietig word. Dit sal geensins die uitkoms van die nadoodse ondersoek, -prosesse of die dienste wat deur Forensiese Patologie Dienste gelewer word, beïnvloed nie.

Toestemming is heeltemal vrywillig. U mag weier of enige tyd tydens die studie-tydperk onttrek.

Toestemming gee of weier?
U deelname is heeltemal vrywillig en sal u niks kos nie (geen geldelike implikasies nie). U het die reg om te weier en om die studie enige tyd te verlaat sonder om 'n rede te verskaf, sonder enige nadelige gevolge. Indien u gewillig is om met die studie te help en toestemming te gee, sal u gevra word om 'n toetsemming vorm te teken.

Weereens U mag die studie enige tydperk verlaat, Indien die bloedmonsters alreeds ontleed/getoets is wanneer u besluit om te onttrek, sal die resultate vanaf die veilige databasis verwyder word. Dit beteken dat geen latere navorsers toegang tot dit sal hê nie.
U besluit om deel te neem of te weier, sal nie die dienste wat deur die Forensisese Patologie Dienste aan u gelewer word, beïnvloed nie.

Wat gebeur met die toetse se uitslae/resultate?
Die resultate van die toetse sal aan die Forensiese Patoloog, wie die nadoodse ondersoek tydens haar/sy roetine nadoodse ondersoeke gedoen het, gegee word.

Die uitslae sal nie aan die naasbestaandes of familie deur middel van hierdie studie gegee word nie.
Die inligting wat ingesamel word, kan deur navorsers gebruik word om artikels in navorsings-joernale te publiseer en/of tydens vergaderings of konferensies voor te dra.

Hoe sal die bloedmonsters gestoor of in toekomstige studies gebruik word?
Die bloedmonsters sal by die Forensiese Toksikologie Eenheid laboratorium van die Afdeling van Forensiese Medisyne en Toksikologie aan die Universiteit van Kaapstad (UK) gestoor word. Die stoor en hantering sal volgens standaard bedryfsprosedures vir biologiese monsterberging, as deel van hul gewone operasionele gevallewerk of nie-gevallewerk prosesse, gedoen word.

Neem asseblief kennis dat die tegnologie in die labotoriums voortdurend verbeter, so met tyd mag daar dalk nuwer labotorium tegnieke ontwikkel word wat die navorsers kan gebruik. U gaan dus ook gevra word om in te stem dat die biologiese materiaal, wat tydens hierdie studie van die oorledene geneem is, vit n tydperk van 5 jaar gestoor mag word, en vir nuwer tegnologiese gevorderde metodes gebruik mag word (as en wanneer dit beskikbaar raak).
U is egter onder geen verpligting om aan toekomstige studies ook deel te neem nie. Om deelname aan toekomstige studies te weier, sal geensins die uitkoms van die nadoodse ondersoek of hierdie navorsings projek beïnvloed nie.

Etiese goedkeuring:
Die biologiese bloedmonsters sal nie vir enige navorsing gebruik word nie, tensy die studie deur die Gesondheidsnavorsingsetiekkomitee van die Universiteit van Kaapstad goedgekeur is.
Hierdie komitee is verantwoordelik om die regte van mense wie vrywillig aan navorsingstudies deelneem te beskerm.

Indien u instem om deel te neem aan hierdie studie, lees asseblief deur die toestemming vorm aandagtig deur en teken dit. Kies asseblief "Ja" of "Nee" deur die toepaslike blokkie met 'n ✓ te merk, nadat u elke sin gelees het.

Dit maak nie saak of u wat u besluit nie. U keuse sal nie vir u of u familie op enige manier benadeel nie. Indien u enige vrae of 'n verwysing na 'n Berading Sentrum of Sielkundige ondersteuning nodig het, moet asb. nie huiwer om met die persoon wat u toestemming verkry te praat en te vra nie.
Indien u nog enige verdere vra in verband met hierdie studie het, kontak asb.:

- Navorser: Dr. Liza Clegg | Tel: 021 406 6192 | Epos: liza.clegg@uct.ac.za
- Studieleier: Dr Gavin Kirk | Tel: 021 406 6192 | Epos: gavin.kirk@uct.ac.za
- Gesondheidsnavorsingsetiekkomitee Voorsitter: Prof. Marc Blockman | Tel: 021 406 6492 | Epos: hrec-enquiries@uct.ac.za

TOESTEMMING VORM

Ek, _____,
(volle name en van van die oorledene se naasbestaande)

die eggenoot/eggenote/maat(partner)/volwasse kind/ouer/voog/volwasse broer/volwasse suster/ander (omkring asb. wat u verhouding tot die oorledene was. Indien "ander", spesifiseer:_____)

van die oorledene met opname nommer WC11/_____/_____, stem saam dat:

	Ja	Nee
1. Ek het die inligtingstuk gelees en al die inligting daarin verstaan.		
2. Die doel van hierdie studie en die prosedures is aan my verduidelik.		
3. Ek verstaan dat deelname vrywillig is.		
4. Ek is bewus dat ek enige tyd (voor of tydens die studie) mag onttrek (my toestemming terugtrek), sonder om 'n rede te verskaf of dat dit enige nadelige gevolge sal hê.		
5. Die identiteit van die oorledene en familielede sal beskerm word en anonym sal bly.		

Ek gee toestemming dat:

	Ja	Nee
Drie (3) bloedmonsters van die oorledene vir toksikologie analise/toetse van die oorledene geneem mag word.		
Die bloedmonsters om by die Universiteit van Kaapstad vir 'n tydperk van 5 jaar gestoor te word, waarna dit toepaslik vernietig sal word.		
Die gestoorde bloedmonsters slegs vir verdere navorsingstudies wat deur die Gesondheidsnavorsingsetiekkomitee van die Universiteit van Kaapstad hersien en goedgekeur is, gebruik mag word.		

Ek verstaan dat:

	Ja	Nee
Die behandeling en hantering van die biologiese materiaal van die oorledene volgens die Universiteit van Kaapstad se Gesondheidsnavorsingsetiekkomitee se riglyne sal plaasvind.		
Die wetenskaplike laboratoriums onder verpligting is om mediese konfidensialiteit te respekteer.		
Ek enige tyd van die studie mag onttrek en my toestemming terugtrek, en dat ek dan die hoof navorsers van my besluit om te onttrek, moet laat weet.		
Die navorsing wat op die bloedmonsters gedoen is, gepubliseer mag word, maar dat die persoonlike inligting van die oorledene of die oorledene se familie nie bekend gemaak sal word nie.		

Parafeer: Toestemming gevra deur:
Toestemming gee deur:
Getuie:

Persoon wat toestemming vra se volle naam/name en van

Handtekening van persoon wat toestemming vra | Datum

Persoon wat toestem tot die neem van bloedmosters vir hierdie studie se volle naam/name en van

Persoon wat toestemming gee se handtekening | Datum

Getuie se handtekening | Datum

Duimafdruk van die oorledene se naasbestaande:

5. Informed consent information sheet and form: isiXhosa

IPHEPHA LOLWAZI LWEZALAMANI ZABATHATHI-NXAXHEBA

Iprojekthi yoPhando:
Uphando lokusasazeka kwamachiza emzimbeni emva kokufa kwiqela labantu abasweleka ngendlela engeyoyemvelo eKapa, eMzantsi Afrika.

Umphathi: UGqr. G. Kirk
Oosekela mthathi: B. Davies and K. Hlela
Umphandi: UGqr. L. Clegg

Singoobani?
Uyamenywa ukuba uthathe inxaxheba kuphononongo lwentsebenziswano neCandelo *leForensic Medicine* kunye ne*Toxicology* kwiYunivesithi yaseKapa (*UCT*) kunye nendawo yokugcina izidumbu eSalt River. Iza kuqhutywa nguGqirha Liza Clegg noGqirha Gavin Kirk, kunye neengcali zetyhefu uBronwen Davies noKathrina Hlela.

Injongo yophando lophando kukuzama ukuqonda ukuba isixa sechiza sitshintsha njani na egazini emva kokuba umntu eswelekile. Le fomu icacisa into oya kucelwa ukuba uyenze ukuba uthatha isigqibo sokuthatha inxaxheba kolu phononongo. Nceda uyifunde ngononophelo kwaye uzive ukhululekile ukubuza nayiphi na imibuzo phambi kokuba uthathe isigqibo malunga nokuthatha inxaxheba.

Iinkcukacha zemvelaphi:
Xa umntu esweleka kwaye kurhaneleka ukuba usweleke ngendlela engeyoyemvelo, kufuneka utyando lwesidumbu lwenziwe ngugqirha ogunyazisiweyo wecandelo lezempilo eNtshona Koloni, kwiSebe lezeMpilo, kwindawo yokugcina izidumbu yeNkonzo ye*Forensic Pathology* (*FPS*). Njengenxalenye yenkqubo yesiqhelo yokuhlolwa kwesidumbu, ugqirha uya kuqokelela igazi elizakuvavanywa ngenjongo yokufumanisa unobangela wokubhubha komntu lowo uhlolwayo. Olunye lwamaphando abalulekileyo enziwayo luhlalutyo lwe-*toxicology* ukufumanisa ukuba umntu ebetye amachiza okanye ityhefu phambi kokufa. Oku kubhenceka kusenokuba kwenzeke ngenjongo okanye ngempazamo. Uvavanyo lobukho bamachiza okanye ityhefu lwenziwa kuninzi lwezidumbu njengeenkonzo zesiqhelo. Ukuba kukho okufunyenweyo, kunokuncedisa ugqirha ekufumaneni unobangela wokufa.

Nangona kunjalo, umthamo wechiza egazini unokutshintsha emva kokuba umntu efile. Amaxesha amaninzi utyando lwesidumbu lwenzeka kwiintsuku ezimbalwa emva kokuba umntu engenisiwe kumzi wogcino-zidumbu. Oku kunokwenza kube nzima ukutolikwa konobangela wokufa. Ukuba isampuli yegazi ithathwa ngexesha lokungeniswa kwindawo yokugcina izidumbu (phambi koqhaqho), isixa sechiza esisegazini siyafana nesi sexesha lokufa. Oku kunokunika umfanekiso ongcono wendima yamachiza ekufeni.

Yintoni injongo yesi sifundo?

Kolu phononongo, sijolise ekuchazeni utshintsho kwisixa seziyobisi egazini, phakathi kokungeniswa kwindawo yokugcina izidumbu kunye noqhaqho lwesidumbu (igazi elithathwe kwiindawo ezahlukeneyo - emlenzeni nasembindini womzimba). Olu lwazi ekugqibeleni luya kuphucula iinkqubo zovavanyo kwindawo yogcino-zidumbu kwixesha elizayo.

Kutheni kusenziwa oludliwondlebe?

Ucelwe ukuba ube nodliwano-ndlebe kuba uze kwindawo yogcino-zidumbu eSalt River emva kokuba ilungu losapho lwakho/isizalwane sakho siswelekile ngendlela engeyoyamvelo. Ukuze senze olu phononongo, kufuneka sicele ukuqokelela iisampuli zegazi ezintathu (3) kwisizalwane sakho.

Inkqubo

Igazi (elingange-3 mL) liya kuqokelelwa yi-*Forensic Pathologist* egunyazisiweyo ukusuka kumthambo wegazi osemlenzeni xa umfi engeniswe kwigumbi logcino-zidumbu eSalt River. Iisampulu zegazi (kwakhona ezingange-3 mL) ezongezelelweyo ezisuka entliziyweni kunye nomthambo wegazi emlenzeni ziya kuqokelelwa ngexesha lokuxilongwa kwesidumbu. Ukuqokelelwa kwezi sampuli akuyi kuchaphazela iinkqubo eziqhelekileyo zovavanyo kwaye akuyi kubangela nayiphi na ingozi eyongezelelweyo kumzimba oxilongwayo. Ezi sampuli zegazi ziya kuvavanyelwa amachiza aqhelekileyo achongwe eKapa yi*Forensic Toxicology Unit* labhoratri yase-*FPS/UCT*.

Igama lelungu losapho lwakho alizukwaziswa. Endaweni yoko, iisampulu kunye nedatha ye-*toxicology* eveliswayo ayiyi kuchazwa, okuthetha ukuba naziphi na izazisi ziya kutshintshwa ngamanani okanye iisimboli.

Imfihlo yeesampulu kunye nedatha iya kugcinwa ngeendlela ezilandelayo:

1. Wonke umntu obandakanyekayo kwiprojekthi utyikitya isivumelwano semfihlo njengenxalenye yenkqubo yolawulo lomgangatho we-*FTU*.
2. Ngabaphandi abaphambili kuphela abaya kuba nokufikelela kwiziko ledatha.
3. Zonke iisampuli, idatha kunye neziphumo ezenziweyo ziya kugcinwa kwispredishithi esikhuselwe nge-*password*.
4. Iziphumo zolu phando zinokupapashwa okanye zinikezelwe ezintlanganisweni kodwa izazisi zabathathi nxaxheba aziyi kuchazwa.
5. Ukuthatha kwakho inxaxheba kuya kugcinwa kuyimfihlo.

Kwenzeka ntoni xa ufika kumzi wogcino-zidumbu emva kokuba uqhaqho lwenziwe?

Uvavanyo lwesidumbu kunye nokuqokelelwa kweesampulu ngokwesiqhelo kwenzeka emva kokufumana imvume kunye nokufanisa umzimba sisizalwane. Nangona kunjalo, ngamanye amaxesha utyando lwenziwa ngaphambi kokuba umzimba ufanisiwe, into ethi udliwanondlebe lokufumana imvume yakho lwenziwa emva kokuba iisampuli sele ziqokelelwe. Kwiimeko ezinjalo, iisampulu ziya kuqokelelwa kwaye zigcinwe de kufunyanwe imvume kwisalamane. Kwiimeko apho imvume ingafunyanwanga okanye imvume yaliwe, iisampulu ezithathiweyo ziya kutshatyalaliswa. Oku akuyi kuchaphazela isiphumo

senkqubo yovavanyo lwesidumbu, okanye iinkonzo ezinikezelwa yi-*FPS* (IiNkonzo ze-*Forensic Pathology*). Imvume yakho yeyokuzithandela, kwaye ungala ukuthatha inxaxheba nangaliphi na ixesha.

Ukwenza ukhetho lwakho

Ukuthatha kwakho inxaxheba kungokokuzithandela kwaye akuyi kuxabisa nantoni na. Ukhululekile ukwala ukuthatha inxaxheba nangaliphi na ixesha, nangasiphi na isizathu, ngaphandle kwesohlwayo. Ukuba uyavuma ukunika imvume, uya kucelwa ukuba usayine ifomu yemvume. Ungayirhoxisa imvume yakho kuphononongo nangaliphi na ixesha, ngaso nasiphi na isizathu. Ukuba isampuli sele ihlalutyiwe ngexesha utshintsha ingqondo yakho, iziphumo zakho ziya kususwa kwisiseko sedatha ekhuselekileyo. Oko kuthetha ukuba akukho baphandi abongezelelweyo abanokufumana idatha.

Isigqibo sakho malunga nokunikezela ngemvume asisayi kuchaphazela naziphi na iinkonzo ozinikwayo yi-*FPS* (IiNkonzo ze*Forensic Pathology*).

Kwenzeka ntoni kwiziphumo?

Iziphumo ziya kunikwa i-*Forensic Pathologist* eyenze utyando njengenxalenye yophando lwakhe lwesiqhelo. Iziphumo aziyi kunikwa izalamane zomfi. Ulwazi oluqokelelweyo lunokupapashwa ngabaphandi kwiijenali zophando kunye/okanye kwiintlanganiso okanye iinkomfa.

Iisampulu ziya kugcinwa njani kwaye zisetyenziswe njani kwizifundo ezizayo?

Iisampulu ziya kugcinwa kwilebhu ye*Forensic Toxicology Unit* esekwe kwiCandelo le*Forensic Medicine* ne*Toxicology*, e-*UCT*. Ukugcinwa kunye nokuphathwa kweesampulu kuya kwenziwa ngokusekelwe kwiinkqubo zokusebenza eziqhelekileyo zokugcinwa kwesampulu yebhayoloji njengenxalenye ye-*casework* yelebhu.

Njengoko itekhnoloji kwiilebhu iphucuka, ekuhambeni kwexesha kunokubakho iindlela ezintsha ezinokusetyenziswa ngabaphandi. Uyakucelwa kwakhona ukuba uvumele iisampulu eziqokelelweyo zisetyenziswe kwezi ndlela zintsha zetekhnoloji ukuba ziye zaba khona. Nangona kunjalo, awunyanzelekanga ukuba uthathe inxaxheba kwizifundo zexesha elizayo. Ukungathabathi nxaxheba kwizifundo ezizayo akuyi kuchaphazela iziphumo zolutyando okanye le projekthi yophando. Ukuba uyasivuma esi sifundo, kodwa awufuni ukuthatha inxaxheba kwizifundo ezilandelayo, nceda ukhethe u'Hayi' kulamacandelo alandelayo:

- Vumelana ukuba iisampulu zingagcinwa kwiDyunivesithi yaseKapa isithuba seminyaka eli-5, emva koko ziya kulahlwa ngokufanelekileyo..
- Iisampulu zegazi ezigciniweyo zinokusetyenziswa kuphela kuphando olongezelelweyo oluye lwaphononongwa kwaye lwamkelwa yiYunivesithi yaseKapa, kwi*Faculty of Health Sciences, Human Research Ethics Committee*.

Imvume yokuziphatha:

Iisampulu aziyi kusetyenziselwa kulo naluphi na uphando ngaphandle kokuba uphononongo luvunyiwe yiYunivesithi yaseKapa, kwi*Faculty of Health Sciences, Human Research Ethics Committee*. Le komiti inoxanduva lokukhusela amalungelo abantu abavolontiyayo ekuthatheni inxaxheba kwizifundo.

Ukuba uyavuma ukuthatha inxaxheba kolu phononongo, nceda ufunde ifomu yemvume ulandelise ngokutyikitya. Emva kokufunda isivakalisi ngasinye; nceda uphawule ibhokisi ethi Ewe okanye Hayi. Nokuba ukhethe ukuthini na, oko akuyi kuchaphazela kakubi wena okanye ilungu lentsapho yakho elingasekhoyo nangayiphi na indlela. Ukuba unemibuzo okanye ufuna ukuthunyelwa kwiziko lentlungu okanye inkxaso yengqondo nceda ubuze lomntu othatha imvume. Ukuba uneminye imibuzo malunga nophononongo, nceda uqhagamshelane no:

Umphathi: UGqr. Gavin Kirk | Umnxeba: 021 406 6192 | Imeyile: gavin.kirk@uct.ac.za
Umphandi: UGqr. Liza Clegg | Umnxeba: 021 406 6192 | Imeyile: liza.clegg@uct.ac.za
Usihlalo we*Human Research Ethics Committee*, Njingalwazi Marc Blockman | Umnxeba: 021 406 6492 | Imeyile: hrec-enquiries@uct.ac.za

IFOMU YEMVUME

Mna, _____ (igama eliphelelelyo lesizalwane), iqabane/oyena umntwana/umzali/umgcini/oyena bhuti/oyena dade (yenza isangqa kubudlelwane obufanelekileyo) walowo oswelekileyo onenombolo ehti WC11/_____/_____.

Ndiyaqinisekisa ukuba:

	Yes	No
1. Ndilufundile ndaluqonda ulwazi olunikwe kwiphepha lolwazi		
2. Ndazisiwe ngenjongo yolu phando kunye neenkqubo.		
3. Ndiyaqonda ukuba ukuthatha inxaxheba kukuzithandela.		
4. Ndiyazi ukuba ndingarhoxa kuphononongo nangaliphi na ixesha ngaphandle kwesizathu okanye isohlwayo		
5. Imfihlo yesazisi iya kugcinwa kwaye umfi okanye usapho lwam aluyi kuvezwa		

Ndiyavuma ukuba:

	Ewe	Hayi
Iisampulu zegazi ezintathu zingaqokelelwa kumfi ukuze kuhlalutywe ngokwakwa-*toxicology*.		
Iisampulu zingagcinwa kwiDyunivesithi yaseKapa isithuba seminyaka eli-5, emva koko ziya kulahlwa ngokufanelekileyo.		
Iisampulu zegazi ezigciniweyo zinokusetyenziswa kuphela uphando olongezelelweyo oluthe lwaphononongwa lwaza lwamkelwa yiYunivesithi yaseKapa, kwi*Faculty of Health Sciences, Human Research Ethics Committee*.		

Ndiyaqonda ukuba:

	Ewe	Hayi
Ukuphathwa kunye nolawulo lweesampulu zebhayoloji zesalamani sam esibhubhileyo kuya kuhambelana nezikhokelo zeYunivesithi yaseKapa, kwi*Faculty of Health Sciences, Human Research Ethics Committee*.		
Iilebhu zenzululwazi ziphantsi koxanduva lokuhlonipha ubumfihlo bezonyango.		
Ndiyakwazi nangaliphi na ixesha ukurhoxisa imvume yam kwaye kufuneka ndazise umphandi ophambili ngesigqibo sam sokurhoxa.		
Uphando olwenziwe ngezi sampuli lunokupapashwa, kodwa umfi okanye usapho lwakhe akayi kuchazwa.		

Igama eliphelelelyo lomntu othatha imvume

Utyikityo lomntu ofumana imvume Umhla

Igama eliphelelelyo lomntu ogunyazisa imvume Umhla
yokuqokelela iisampulu kutyando ukuze zisetyenziswe kuphononongo lophando

Utyikityo lwengqina Umhla

Ubhontsi weqabane/oyena mntwana/umzali/umgcini/oyena bhuti/oyena dade womfi:

6. Full results tables

		RESULTS (mg/L, except *ug/L)													
Case Nr	Sample	ACM	ACE	AMP	BEN	DZP	DIP	MDA	MDM	MTD	MET	MEQ	MOR	NCT	TRM
2	Admission - F1	-	0.590	-	-	-	-	-	-	-	-	-	-	-	-
	Autopsy - F2	-	1.226	-	-	-	-	-	-	-	-	-	-	-	-
4	Autopsy - C	-	-	<0.02	-	-	-	-	-	-	0.0489	0.1764	-	0.0298	-
	Admission - F1	-	-	<0.01	-	-	-	-	-	-	0.0391	0.1606	-	0.0164	-
	Autopsy - F2	-	-	<0.02	-	-	-	-	-	-	0.0561	0.1808	-	0.0333	-
6	Autopsy - C	-	8.196	0.0285	-	-	-	-	-	-	0.4353	1.2570	-	-	0.0283
	Admission - F1	-	5.142	<0.01	-	-	-	-	-	-	0.2502	0.6599	-	-	<0.02
	Autopsy - F2	-	7.408	0.0237	-	-	-	-	-	-	0.2916	1.0413	-	-	0.0295
13	Autopsy - C	-	-	-	-	-	-	-	-	-	-	0.0768	-	-	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	0.0788	-	-	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	<0.05	-	-	-
14	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	0.0144	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	0.0103	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	-	-	<0.01	-
10	Autopsy - C	-	-	0.0906	-	<0.01	<0.04	-	-	-	1.1394	0.9132	-		-
	Admission - F1	-	-	0.0271	-	<0.02	<0.01	-	-	-	0.6842	0.9326	-		-
	Autopsy - F2	-	-	0.0553	-	<0.02	<0.04	-	-	-	0.9098	1.1711	-		-
19	Autopsy - C	-	-	-	0.2336	-	-	-	-	-	-	1.2396	-		-
	Admission - F1	-	-	-	0.2607	-	-	-	-	-	-	0.5801	-		-
	Autopsy - F2	-	-	-	0.1619	-	-	-	-	-	-	0.3994	-		-
30	Autopsy - C	-	-	0.1033	-	-	-	-	-	-	0.5921	1.6263	0.0287		-
	Admission - F1	-	-	0.0326	-	-	-	-	-	-	0.2966	1.116	<0.01	-	-
	Autopsy - F2	-	-	0.0515	-	-	-	-	-	-	0.3906	1.1958	0.0447	-	-
25	Autopsy - C	-	-	0.0834	-	-	-	-	-	-	0.1686	-	-	-	-
	Admission - F1	-	-	0.0274	-	-	-	-	-	-	0.0591	-	-	-	-
	Autopsy - F2	-	-	0.0470	-	-	-	-	-	-	0.1168	-	-	-	-
34	Autopsy - C	-	-	0.4847	-	-	-	-	-	-	5.4592	0.0866	-	-	-
	Admission - F1	-	-	0.0658	-	-	-	-	-	-	0.7898	0.0535	-	-	-
	Autopsy - F2	-	-	0.1695	-	-	-	-	-	-	2.0653	0.0695	-	0.0200	-
36	Autopsy - C	-	-	<0.02	-	0.0767	-	-	-	-	0.0756	1.6445	-	-	-
	Admission - F1	-	-	<0.01	-	0.1145	-	-	-	-	0.0258	1.8373	-	-	-
	Autopsy - F2	-	-	<0.02	-	0.0741	-	-	-	-	0.0481	1.9264	-	-	-
3	Autopsy - C	-	-	0.1313	-	-	0.0432	-	-	-	0.8653	0.9677	-	-	-
	Admission - F1	-	-	0.0265	-	-	<0,01	-	-	-	0.3096	0.6044	-	-	-
	Autopsy - F2	-	-	0.0279	-	-	<0,01	-	-	-	0.2441	0.5186	-	-	-
40	Autopsy - C	-	-	0.0597	-	-	-	-	-	-	0.4964	2.3148	-	-	-
	Admission - F1	-	-	0.0312	-	-	-	-	-	-	0.311	2.4289	-	-	-
	Autopsy - F2	-	-	0.0326	-	-	-	-	-	-	0.2541	1.9744	-	-	-
46	Autopsy - C	-	18.73112	-	-	-	-	-	-	-	-	-	-	-	-
	Admission - F1	-	23.520	-	-	-	-	-	-	-	-	-	-	-	-
	Autopsy - F2	-	15.83857	-	-	-	-	-	-	-	-	-	-	-	-
52	Autopsy - C	-	0.4291	-	-	-	-	-	-	-	-	-	-	-	-
	Admission - F1	-	0.2749	-	-	-	-	-	-	-	-	-	-	-	-
	Autopsy - F2	-	0.2374	-	-	-	-	-	-	-	-	-	-	-	-
42	Autopsy - C	-	1.1955	-	-	-	-	-	-	-	-	-	-	-	-
	Admission - F1	-	0.4241	-	-	-	-	-	-	-	-	-	-	-	-
	Autopsy - F2	-	0.4295	-	-	-	-	-	-	-	-	-	-	-	-
44	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
62	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	0.0156	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	0.0227	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	-	-	0.0169	-
57	Autopsy - C	-	-	0.1479	-	-	0.3262	-	-	-	0.8673	1.7605	-	-	-
	Admission - F1	-	-	0.1314	-	-	0.2506	-	-	-	0.7595	1.4505	-	-	-
	Autopsy - F2	-	-	0.0773	-	-	0.1846	-	-	-	0.5719	1.6059	-	-	-

Case Nr	Sample	ACM	ACE	AMP	BEN	DZP	DIP	MDA	MDM	MTD	MET	MEQ	MOR	NCT	TRM
61	Autopsy - C	-	-	0.0764	-	-	-	-	-	-	0.4458	-	-	-	-
	Admission - F1	-	-	0.0309	-	-	-	-	-	-	0.2146	-	-	-	-
	Autopsy - F2	-	-	0.0289	-	-	-	-	-	-	0.2249	-	-	-	-
66	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	0.0109	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	0.0278	-
	Autopsy - F2	-	0.3641	-	-	-	-	-	-	-	-	-	-	0.0236	-
67	Autopsy - C	-	-	0.0273	-	-	-	-	-	-	0.1198	0.1096	-	-	-
	Admission - F1	-	-	<0.02	-	-	-	-	-	-	0.0731	0.1275	-	-	-
	Autopsy - F2	-	-	0.0588	-	-	-	-	-	-	0.2914	0.1995	-	-	-
70	Autopsy - C	-	-	0.0585	-	-	-	-	-	-	0.8384	1.3748	-	-	-
	Admission - F1	-	-	<0.01	-	-	-	-	-	-	0.1894	0.9511	-	-	-
	Autopsy - F2	-	-	<0.02	-	-	-	-	-	-	0.2212	0.8865	-	-	-
86	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	0.0450	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	0.0546	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	-	-	0.0267	-
83	Autopsy - C	-	-	-	0.0218	-	-	0.0613	4.0612	-	-	-	-	0.0662	-
	Admission - F1	-	-	-	0.0241	-	-	0.0126	0.4897	-	-	-	-	0.0837	-
	Autopsy - F2	-	-	-	0.0295	-	-	0.0157	0.4367	-	-	-	-	0.0419	-
78	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	0.0447	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	0.0607	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	-	-	<0,01	-
79	Autopsy - C	-	49.52079	-	-	-	-	-	-	-	-	-	-	-	-
	Admission - F1	-	39.72536	-	-	-	-	-	-	-	-	-	-	-	-
	Autopsy - F2	-	44.00781	-	-	-	-	-	-	-	-	-	-	-	-
76	Autopsy - C	-	12.391	-	-	-	-	-	-	-	-	0.1314	-	-	-
	Admission - F1	-	9.0771	-	-	-	-	-	-	-	-	0.1915	-	-	-
	Autopsy - F2	-	10.063	-	-	-	-	-	-	-	-	0.1853	-	-	-
91	Autopsy - C	-	-	0.0723	-	0.0355	0.0916	-	-	-	0.7649	1.8724	-	-	-
	Admission - F1	-	-	<0,02	-	0.0315	<0,01	-	-	-	0.1823	1.6337	-	-	-
	Autopsy - F2	-	-	0.0369	-	<0,02	<0,04	-	-	-	0.2775	1.7038	-	-	-
73	Autopsy - C	-	0.9821	-	-	-	-	-	-	-	-	-	-	-	-
	Admission - F1	-	0.2312	-	-	-	-	-	-	-	-	-	-	-	-
	Autopsy - F2	-	0.6104	-	-	-	-	-	-	-	-	-	-	-	-
77	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	0.0541	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	0.0921	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	-	-	0.0558	-
95	Autopsy - C	-	-	0.1149	-	0.0477	-	-	-	-	1.9246	1.2922	-	-	-
	Admission - F1	-	-	0.0221	-	0.0289	-	-	-	-	0.3121	0.8708	-	-	-
	Autopsy - F2	-	-	0.0240	-	0.0266	-	-	-	-	0.4010	0.9230	-	-	-
94	Autopsy - C	-	0.2208	-	-	-	-	-	-	-	-	-	-	-	-
	Admission - F1	-	0.1752	-	-	-	-	-	-	-	-	-	-	-	-
	Autopsy - F2	-	0.2888	-	-	-	-	-	-	-	-	-	-	-	-
89	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	0.0217	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	0.0195	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	-	-	0.0147	-
90	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	0.0118	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	0.0239	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	-	-	0.0112	-
98	Autopsy - C	-	-	0.0314	-	-	-	-	-	-	0.2782	1.4759	-	-	-
	Admission - F1	-	-	0.0303	-	-	-	-	-	-	0.3174	2.3343	-	-	-
	Autopsy - F2	-	-	0.0229	-	-	-	-	-	-	0.2965	2.1772	-	-	-
96	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	0.0162	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	0.0472	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	-	-	0.0134	-
103	Autopsy - C	-	-	0.3559	-	-	-	-	-	-	1.8158	1.2215	-	-	-
	Admission - F1	-	-	0.0493	-	-	-	-	-	-	0.5110	1.1962	-	-	-
	Autopsy - F2	-	-	0.0950	-	-	-	-	-	-	0.5245	0.9728	-	-	-
102	Autopsy - C	-	0.2620	-	-	-	-	-	-	0.1417	0.0415	-	<0,01	-	0.0895
	Admission - F1	-	0.1492	-	-	-	-	-	-	0.1265	0.0342	-	<0,01	-	0.0723
	Autopsy - F2	-	0.2333	-	-	-	-	-	-	0.0798	0.0317	-	0.0118	-	0.0809

Case Nr	Sample	ACM	ACE	AMP	BEN	DZP	DIP	MDA	MDM	MTD	MET	MEQ	MOR	NCT	TRM
104	Autopsy - C	-	-	-	-	0.0275	-	-	-	-	0.1507	-	-	-	-
	Admission - F1	-	-	-	-	0.0313	-	-	-	-	0.0489	-	-	-	-
	Autopsy - F2	-	-	-	-	<0.01	-	-	-	-	0.0858	-	-	-	-
107	Autopsy - C	-	-	0.1794	-	-	-	-	-	-	1.7441	0.7789	-	-	-
	Admission - F1	-	-	0.2459	-	-	-	-	-	-	1.4575	0.7372	-	-	-
	Autopsy - F2	-	-	0.0223	-	-	-	-	-	-	0.3017	0.7391	-	-	-
115	Autopsy - C	-	-	0.0897	-	-	-	-	-	-	0.8423	0.2969	-	0.0231	-
	Admission - F1	-	-	0.0733	-	-	-	-	-	-	0.8676	0.2532	-	0.0258	-
	Autopsy - F2	-	-	0.0675	-	-	-	-	-	-	0.5449	0.1912	-	-	-
120	Autopsy - C	-	-	0.2447	-	-	0.1022	-	-	-	2.4644	1.5959	-	0.0257	-
	Admission - F1	-	-	0.0564	-	-	<0.04	-	-	-	0.4948	1.9268	-	0.0404	-
	Autopsy - F2	-	-	0.0453	-	-	<0.04	-	-	-	0.4758	1.8868	-	0.0194	-
127	Autopsy - C	-	<0,1	0.4528	-	-	-	-	-	-	1.7265	0.7853	0.4696	-	-
	Admission - F1	-	<0,1	0.0298	-	-	-	-	-	-	0.2282	0.7439	0.0351	-	-
	Autopsy - F2	0.0118	0.5820	0.1058	-	-	-	-	-	-	0.6967	0.7620	2.0333	-	-
162	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	0.0534	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	0.0901	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	-	-	0.2410	-
146	Autopsy - C	-	-	-	-	-	-	-	-	-	-	-	-	0.0368	-
	Admission - F1	-	-	-	-	-	-	-	-	-	-	-	-	0.1425	-
	Autopsy - F2	-	-	-	-	-	-	-	-	-	-	-	-	0.0601	-
166	Autopsy - C													0.0535	
	Admission - F1													0.0881	
	Autopsy - F2													0.034	
151	Autopsy - C											<0,025		<0,01	
	Admission - F1											0.0544		0.0131	
	Autopsy - F2											<0,05		<0,005	
167	Autopsy - C													0.0361	
	Admission - F1													0.0404	
	Autopsy - F2													0.0554	
160	Autopsy - C		0.1537												
	Admission - F1		<0,02												
	Autopsy - F2		0.1132												
171	Autopsy - C		0.6605	0.0765			0.052				0.2406	2.2206			
	Admission - F1		0.6334	0.0352			0.04				0.1872	1.9383			
	Autopsy - F2		1.8497	0.0719			0.0419				0.3802	1.6909			
168	Autopsy - C			0.0993							0.7493	0.6522			
	Admission - F1			0.0487							0.2301	0.9016			
	Autopsy - F2			0.076							0.5787	0.8066			
172	Autopsy - C			0.1502			0.0568				2.3082	0.7547			
	Admission - F1			0.0278			<0,04				0.2796	0.4574			
	Autopsy - F2			0.0434			<0,04				0.4639	0.5175			
164	Autopsy - C														
	Admission - F1													0.015	
	Autopsy - F2													0.01	
188	Autopsy - C			0.1485			0.0735				0.48	1.3535			
	Admission - F1			0.0239			0.015				0.0733	1.6466			
	Autopsy - F2			0.0535			0.0465				0.2248	1.4401			
185	Autopsy - C										0.2239	2.3785			
	Admission - F1										0.0691	2.2205			
	Autopsy - F2										0.0599	1.9574			
197	Admission - F1			0.0341							0.2553	0.481			
	Autopsy - F2			0.0383							0.2674	0.4302			
187	Autopsy - C			0.1232			0.0724				1.1052	2.0026		0.1248	
	Admission - F1			0.0269			<0,01				0.3134	1.0564		0.0333	
	Autopsy - F2			0.1066			0.0728				1.4405	1.0826		0.0237	
192	Autopsy - C			0.4985			0.0456				4.1245	0.8139			
	Admission - F1			0.0654			<0,01				1.1988	0.8527			
	Autopsy - F2			0.1334			<0,01				1.4207	0.7389			
199	Autopsy - C			0.0933		<0,01					0.5855	0.405			
	Admission - F1			0.035		0.0216					0.4662	0.5946			
	Autopsy - F2			0.0602		<0,02					0.3374	0.5617			
149	Autopsy - C			0.1242			0.1346				1.5417	3.4198			
	Admission - F1			0.0387			<0,01				0.2114	1.9021			
	Autopsy - F2			0.163			0.0572				1.9705	2.4222			