Post-mortem toxicological investigations in a paediatric population

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STUDENT NUMBER: LWXANE002

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In partial fulfilment of the requirements for the degree

MPhil (Biomedical Forensic Science)

Division of Forensic Medicine and Toxicology

Department of Pathology

UNIVERSITY OF CAPE TOWN

20 December 2019

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Declaration

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Abstract

In South Africa, little is known about the presence of drugs in infant cases admitted for post-mortem medico-legal examinations, as toxicological investigations are not routinely performed. It was hypothesised that drugs would be detected in sudden and unexpected death of infant (SUDI) cases admitted to Salt River Mortuary (SRM), as infants form a vulnerable population. Biological samples (blood, vitreous humour, urine and hair) were collected from 30 infants who were admitted as SUDI cases to Salt River Mortuary, Cape Town, between 28 May 2019 and 17 October 2019. Samples were screened for at least 750 common drugs using a SCIEX X500R QTOF. Demographic variables, social circumstances and clinical history were recorded from the medico-legal case folder. Of the 30 SUDI cases, drugs were detected in 25 (83 %) cases, with acetaminophen (61 %) and caffeine (54 %) being most prevalent. Methaqualone (32 %) and methamphetamine (11 %), two commonly abused drugs in the Western Cape, were also identified, with the former only present in hair. There were significantly more drugs detected in hair samples compared to the other samples ($p < 0.0001$). Therefore, while challenging in its interpretation, hair analyses provided a wealth of information concerning possible longer-term drug exposure in infants. This was particularly valuable in revealing methaqualone exposure, which may have otherwise gone undetected, and which may indicate an environment of neglect. While the cause of death in most cases was natural (infectious causes) (63 %), next-of-kin seldom declared that their infant exhibited symptoms of illness or that medication was administered prior to death. Therefore, the results of this study illustrate the value of toxicological testing in SUDI cases at SRM, as well as the need to analyse multiple samples. This study provides empirical data to motivate for the SUDI investigation protocol at SRM to include routine toxicological analysis. This is anticipated to add value to the medico-legal investigation as well as add social value to the lives of siblings who may also be at risk for neglect.

Acknowledgements

I would like to thank:

- **My parents –** for their unconditional support (emotionally and financially) throughout my studies the past six years. Thank you for always believing in me and for making my dreams become a reality.
- **My Heavenly Father –** for His provision and strength to finish this degree for His glory.
- **Bronwen Davies and Dr Laura Heathfield** (project supervisors) – for their support, guidance, motivation, and feedback throughout the duration of my research project.
- **Tuscany Botha, Tarryn Haikney, Khilona Natha, Robyn Tichauer –** for their constant support throughout my project, without you I would have not been able to have survived this year.
- **Alicia Evans –** for assisting with the toxicological drug screening procedure.
- **Forensic pathologists and forensic pathology officers at Salt River Mortuary –** for assisting in me recruiting participants in my study and for collecting biological samples for my study.
- **Forensic Pathology Services (Western Cape Department of Health) –** for allowing me to conduct my study at Salt River Mortuary.
- **University of Cape Town –** for funding for my research project.

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List of abbreviations and symbols

%	: percentage
°C	: degrees Celsius
ACN	: acetonitrile
ADME	: absorption, distribution, metabolism and excretion
ARVs	: antiretrovirals
CCM	: cough and cold medication
CDR	: Child Death Review
CNS	: central nervous system
CYP	: cytochrome P450
DCM	: dichloromethane
DSD	: Department of Social Development
FCL	: Forensic Chemistry Laboratory
FPS	: Forensic Pathology Service
g	: gram(s)
GIT	: gastrointestinal tract
HPLC	: high-performance liquid chromatography
HREC	: Human Research Ethics Committee
IA	: intra-arterial
IM	: intramuscular
IMR	: infant mortality rate
IS	: internal standard
IV	: intravenous
L	: litre
LC-QTOF-MS	: liquid chromatography quadrupole time-of-flight mass spectrometry
m	: milli
MP-A	: mobile phase A

MP-B : mobile phase B

NPA : National Prosecution Authority

OTC : over-the-counter

PPI : proton pump inhibitor

REF : reference

rpm : revolutions per minute

RT : retention time

RTI : respiratory tract infection

SACENDU : South African Community Epidemiology Network on Drug Use

SDG : sustainable development goal

SIDS : sudden infant death syndrome

SRM : Salt River Mortuary

SUDI : sudden unexpected death in infants

UCT : University of Cape Town

UI : under investigation

UK : United Kingdom

UN : United Nations

USA : United States of America

WHO : World Health Organisation

µ : micro

Chapter 1: Introduction and literature review

1.1 Background

In 2017, the World Health Organisation (WHO) reported that approximately 75 % (4.1 million) of all deaths in individuals under the age of five years occurred within the first year of life, i.e. during infancy. The risk of an infant dying compared to other children under the age of five years is the highest in the African region (World Health Organisation, 2019a). There has been a global recognition of this problem, which motivated the introduction of the third sustainable development goal (SDG). This goal is aimed at stopping preventable deaths in children under the age of five and reducing under-five mortality to 25 per 1000 live births by 2030 (United Nations, 2015).

Infants form part of a vulnerable population in terms of early morbidity and mortality. One reason for this is their higher risk for exposure to a wide range of xenobiotics (foreign substances that may enter the body of an organism and produce beneficial and/or toxic effects) (Twilley & Lall, 2014; Soucek, 2017). These may include drugs of abuse, prescription and/or over-the-counter (OTC) medication, or household agents, such as bleach, paraffin, and pesticides. Rimsza and Newberry (2008) highlighted that the detection of OTC medication (such as pseudoephedrine, antihistamine or dextromethorphan-based cough and cold medication (CCM)) in infants should raise suspicion and their possible contribution to death should not be ignored (Rimsza & Newberry, 2008). Infants are also at risk for exposure through second-hand smoke and through the transfer of certain drugs via breast milk from a nursing mother (Dolinak, 2013; World Health Organisation, 2019b). When an infant dies following exposure to a harmful drug or chemical, forensic toxicological investigations are useful to determine the contribution of the exposure to death (Middleberg, 2014). Unless specified, any reference to ‘drugs’ in this minor dissertation will refer to, and/or include, drugs of abuse and medication (OTC and prescription).

South Africa has a high infant mortality rate (IMR) (Statistics South Africa, 2018) and falls under the WHO high-risk (African) region for infant deaths (World Health Organisation, 2019a). IMRs between South Africa and developed countries differ notably. In the United Kingdom (UK) and United States of America (USA) for example, IMRs were recorded as 3.8

child deaths per 1000 live births (2016) and 6 child deaths per 1000 live births (2017) respectively (Office for National Statistics, 2018; The World Bank, 2019), whereas South Africa reported an IMR of 36.4 child deaths per 1000 live births (2018) (Statistics South Africa, 2018). Although there has been a decrease in the South African IMR from 2002 to 2018 (Statistics South Africa, 2018), infant deaths remain a burden to the community. In South Africa, toxicological investigations in cases of sudden unexpected death in infants (SUDI), are not yet routine and this poses an issue since the contribution of drugs to death in these cases remains largely unknown. The absence of toxicological testing in many of these cases may thus result in the drawing of incomplete conclusions with regards to the contribution of drugs to death (Dempers et al., 2018).

This chapter reviews the key literature available on the medico-legal death investigation system in South Africa as it pertains to SUDI cases. It reviews the current state of the global burden of disease in infants and factors contributing to this. It comments on infant forensic toxicology and the complexities associated with the interpretation of post-mortem forensic toxicology results in these cases. Finally, the literature review comments on the value of routine toxicological investigations in SUDI cases, which is the focus of this study.

1.2 Sudden unexpected death in infants (SUDI)

If a sudden and unexpected death of an individual under the age of one year occurs, it is referred to as SUDI (Fleming, 2000). The proposed criteria that need to be fulfilled for a death to be classified as SUDI are as follows: the infant was between 7 and 365 days old at the time of death, and death was sudden and unexpected at time of autopsy, or death occurred during an acute illness that was not perceived as life-threatening, or death occurred during an acute illness that lasted less than one day (<24 hours) in a previously healthy individual (or death >24 hours if life was extended by medical procedures), or death was caused by a pre-existing “hidden” disease, or death was caused by any form of poisoning, trauma or accident, which was not obvious at the time of death (Fleming, 2000). These SUDI criteria have not been adopted globally, but it would be ideal if all countries started to use a uniform SUDI classification system to allow for comparative analysis on generated SUDI data and case investigation practice.

SUDI deaths can be classified as explained or unexplained SUDI based on the Avon clinicopathological system (Blair, Byard & Fleming, 2012). If the cause of death remains undetermined after a thorough investigation, the SUDI case is classified as sudden infant death syndrome (SIDS). This thorough investigation must include a full post-mortem examination, ancillary tests, a death scene investigation and evaluation of clinical history (Blair, Byard & Fleming, 2012). Published studies have highlighted the importance of toxicological investigations in these cases to prevent the incorrect classification of a death as SIDS when the detected drugs may have contributed to or caused death (Campbell & Collins, 2001; Langlois et al., 2002; Baker et al., 2003; Bajanowski et al., 2005; Weber et al., 2008).

1.3 Burden of disease in the SUDI population

In 2015, sub-Saharan Africa and Asia recorded the highest levels of under-five mortality (Byass, 2016). This, despite a recorded decline in the overall global burden of disease in children. This illustrates a clear distinction between child and infant morbidity and mortality in developing and developed countries. In 2008, the Western Cape Burden of Disease Reduction Project report indicated that the main causes of childhood mortality and morbidity are malnutrition and infectious diseases (Western Cape Government, 2008). Given that the third SDG is aimed at reducing under-five mortality (United Nations, 2015); investigating risk factors contributing to this high level of mortality, especially in the infant population, is essential.

Death due to respiratory infection is a common cause of death in low- and middle-income countries (World Health Organisation, 2008), with infants being at a particularly high risk (Zar & Ferkol, 2014). Pneumonia, for example, is a common cause of death in children under five years of age and was reported to be one of the global leading causes of death in 2011 in children under five (Walker et al., 2013). Environmental risk factors contributing to respiratory infections in children include exposure to air pollution (including indoor pollution) especially in lower income households, tobacco smoke and poor nutrition amongst others (Torres-Duque et al., 2008; Zar & Ferkol, 2014).

Malnutrition is also often associated with pneumonia-related deaths in children (United Nations Children's Fund, 2013). Risk factors associated with malnutrition include parental neglect or lack of adequate health care (Western Cape Government, 2008). In 2013, the UN

reported that medical attention was sought out for only 80 % of children experiencing symptoms of pneumonia (United Nations Children's Fund, 2013). Not seeking medical attention for sick children may be viewed as child neglect (Matthews & Martin, 2016).

Drug abuse is also a major contributor to the global burden of disease (Wu, 2010). The nature of this contribution may differ between countries and is strongly associated with social development (GBD 2016 Alcohol and Drug Use Collaborators, 2018). In low- and middle-income countries, the extent of drug abuse contributing to the global burden of disease may be higher. Not only does this affect the health state of the drug abuser but also of family members, including children. Child abuse and neglect have been associated with household drug abuse by parents and/or caregivers (Zar & Ferkol, 2014), and this is important to consider in the investigation of SUDI cases.

1.4 South African medico-legal death investigation system

In South Africa, a medico-legal death investigation is required in all cases of suspected unnatural death (Republic of South Africa, 1959). An unnatural death is defined in the Regulations Regarding the Rendering of Forensic Pathology (reg 636) in terms of the National Health Act (2003) and the Health Professions Act (Act 56 of 1974) as any death due to physical or chemical influence (direct or indirect, or related complications); any death as a result of an act of omission or commission, which may be criminal in nature; any procedure-related death; or any death that is sudden, unexpected and/or unexplained or where the cause of death is not apparent (Republic of South Africa, 2007).

Given the above definition, SUDI deaths are mandated to undergo a medico-legal death investigation. The post-mortem examination (which includes an autopsy) is performed by an authorised medical practitioner (e.g. forensic pathologist), working for the Forensic Pathology Service (FPS), to determine the cause of death and also possible circumstances surrounding death (Republic of South Africa, 1959; Dempers et al., 2018). Ancillary investigations are often performed by experts (mostly scientists) on post-mortem specimens and may include histology, microbiology, virology, radiology, toxicology, biochemistry, and metabolic screening. The number of ancillary investigations requested is at the discretion of the medical practitioner (Republic of South Africa, 1959).

Death scene investigations are important and recommended prior to autopsy. During the scene investigation, documentation of all important information is crucial and usually performed through writing notes, drawing diagrams and photography (Demirci & Dogan, 2011). Prahlow (2010) suggested that it is ideal to perform a death scene investigation in all cases if possible, but specifically for all child and infant deaths. Even though death scene investigations are recommended in all SUDI cases (Prahlow, 2010), South Africa is currently lacking a standardised protocol for the death scene investigation of such cases (Du Toit-Prinsloo et al., 2013). This often result in the absence of death scene investigations in such cases. The majority of admitted SUDI cases are classified as natural cause of death after autopsy, while others may be classified as "under investigation" pending histology and/or virology test results(Bennett, 2018). Following a proper death scene investigation, witnesses may use child dolls to recreate the circumstances surrounding an infant death, to give other role players a better idea of what happened at the time of death (Prahlow, 2010).

During the death scene investigation, valuable evidence such as drugs, medication and possible weapons may be found, which may assist in a better understanding of the circumstances surrounding death (Prahlow, 2010). If drugs or chemicals are identified on scene, their collection and subsequent analysis may be especially useful in the interpretation of toxicology results. This is especially important in infant cases where there are positive toxicological results, but limited or no indication of overdose or poisoning from witness testimony alone (Prahlow, 2010).

1.5 Post-mortem toxicology

Toxicology is a discipline that investigates the adverse effects that chemical substances have on living organisms, which includes the measurement and treatment of exposure (Houck & Siegel, 2015). Forensic toxicology involves the application of the science of toxicology to medico-legal cases. Post-mortem toxicology, a discipline of forensic toxicology, involves the collection of biological samples (tissues and/or bodily fluids) from a deceased individual usually at autopsy. These samples undergo toxicological analyses to detect, identify and/or quantify drugs and chemicals to determine whether they contributed to, or caused, death. Post-mortem toxicological analyses therefore play a crucial role in the investigation of unnatural deaths (Dolinak, 2013; Houck & Siegel, 2015; Smith & Bluth, 2016).

In South Africa, the Forensic Chemistry Laboratories (FCL), which fall under the National Government Department of Health, have historically performed routine post-mortem toxicological analyses for FPS cases (Western Cape Government, 2014). In the Western Cape, a continuously increasing caseload, systemic challenges with staffing and funding and previously limited support for the development of forensic toxicology professionals in the country, has likely contributed to the current backlog in forensic toxicology case analysis in these laboratories (Evans, 2016). It is possible that this backlog has inhibited the routine collection and analysis of samples in SUDI cases, especially if there is evidence at the autopsy of a pathological cause of death.

1.5.1 Infant post-mortem toxicology

Infants are more vulnerable to toxic exposures from drugs of abuse, OTC and prescription medication, as well as household products (such as cleaning agents, pesticides and cosmetics), especially when given with malicious intent (Dolinak, 2013). The latter refers to the administration of drugs to an infant with the intent to poison or harm them (Farst & Bolden, 2012). Inadvertent drug exposure may also occur in a number of ways, including inhalation of secondary smoke from smoking parents (or other close relatives or caregivers), the accidental ingestion of drugs or chemicals that are within reach of a young child or ingestion through breast milk (Dolinak, 2013). Infants can be acutely or chronically exposed to drugs, which may - together with other factors, such as weakened immune systems, lower socio-economic and/or poor health statuses - contribute to death. In these cases, a toxicological investigation may be of importance.

When interpreting analytical findings in post-mortem cases, toxicologists need to be cognisant of several factors that can alter concentrations of drugs and/or their metabolites in biological specimens. One of the key factors here is the pharmacokinetics of the drug within the given individual. The drug disposition that occurs prior to death influences the post-mortem concentrations obtained following death and, if not fully considered, may lead to erroneous conclusions in cause and manner of death. This section focuses on the variation of disposition of drugs within infants, placing emphasis on the challenges of interpreting this toxicity within a post-mortem context.

In SUDI cases it is important to keep the developmental differences of infants in mind, including how these differences may alter toxicity if exposed to drugs or chemicals. An in-

depth understanding of infant pharmacokinetics and pharmacodynamics is required in order to accurately interpret quantitative infant post-mortem toxicological results. This is challenging given the limited number of controlled studies investigating drugs in children and/or infants, especially for drugs of abuse. Given that the results generated in this study were qualitative in nature, only a brief overview of differences in pharmacodynamics and- kinetics in infants and adults was provided here.

1.5.2 Infant pharmacokinetics

Altered pharmacokinetic processes in infants due to physiological differences may alter the disposition of drugs in infants. The ante-mortem effects of drugs relate to the pharmacokinetic properties of the drug as well as that of the deceased (Lappas & Lappas, 2016). Other post-mortem influences, such as chemical degradation and post-mortem redistribution may also affect post-mortem drug concentrations but are not the focus of this minor dissertation. Pharmacokinetic parameters provide an observed relationship between administered drug doses and corresponding concentrations in biological samples, such as blood. Given that these parameters are different in infants, they need to be considered in post-mortem toxicological interpretations.

Pharmacokinetics refers to the kinetic process that a drug undergoes within the body once administered through to its elimination. Pharmacokinetics consist of four general processes, namely absorption, distribution, metabolism and excretion (ADME), which is also referred to as the disposition of a drug (Middleberg, 2014). Drugs may be administered through multiple sites, which can greatly affect the extent and rate of drug absorption as well. These processes should all be considered in infant toxicology.

1.5.2.1 Administration

Administration of drugs to infants may occur through either enteral or parenteral routes and can occur both intentionally or unintentionally (Mignani et al., 2013; Lappas & Lappas, 2016). Enteral administration involves the gastrointestinal tract (GIT) and may include oral, sublingual (underneath the tongue) and rectal administration. Parenteral administration may involve dermal or topical (application on skin) application, intravenous (IV), intra-arterial (IA), intramuscular (IM), subcutaneous or intranasal administration, as well as inhalation.

1.5.2.1.1 Parenteral administration

Parenteral administration of drugs to infants can occur both intentionally, when parents administer medication, as well as unintentionally, if accidentally ingested (Tetelbaum et al., 2005; Landrigan & Goldman, 2011). For example, if medication is administered to infants for therapeutic purposes, it may be administered IV or IM (Tetelbaum et al., 2005). Infants may also be unintentionally exposed to the inhalation of secondary smoke for example, or to chemicals in and around the house, some more toxic than others (Tetelbaum et al., 2005; Landrigan & Goldman, 2011).

1.5.2.1.2 Enteral administration

Infants may also be given medication via the oral route, especially in the form of a syrup or other liquid product (Tetelbaum et al., 2005).

One of the major methods of inadvertent drug administration is through transfer via a mother's breast milk, following therapeutic or recreational drug use (Dolinak, 2013).

While breastfeeding of infants is beneficial, it is important to note whether a breastfeeding mother is using drugs or medication and to what extent these drugs are transferred through the breast milk and administered to the infant. The amount of drug ingested by an infant through breast milk is influenced by a number of factors including, but not limited to, the pattern of drug use by the mother, the total volume of breast milk ingested by the infant, as well as a combination of biochemical processes responsible for the maternal metabolism and transfer of drugs (Dolinak, 2013). The produced effects, if any, of ingested drugs are influenced by the following factors: the timing of the dose, oral bioavailability, the relative infant dose (this refers to the dose of drug received by the infant through breast milk relative to the dose administered to the mother) as well as the age of the infant (Hotham & Hotham, 2015).

Certain drugs are therefore contraindicated during lactation and have been reported as such (Committee on Drugs, 2001). The main reason for cessation of breastfeeding when a nursing mother is using any of the listed drugs is to prevent any possible adverse effects that it may have on the infant (Committee on Drugs, 2001). It is however important to keep in mind that the mere presence of a drug in breast milk does not necessarily mean that it produced adverse effects in the infant (Committee on Drugs, 2001; Berlin & Briggs, 2005). On the other hand, detection of a drug that should not be present in an infant may generate concerns of neglect. Ostrea et al. (2004) provides a comprehensive review of drugs that may affect the foetus or

infant following transfer via the breast milk and/or placenta (Ostrea, Mantaring & Silvestre, 2004).

1.5.2.1.3 Placental transfer

Drugs can also be administered to an unborn baby by means of placental transfer (Griffiths & Campbell, 2015). The human placenta is an organ that serves as a connection between mother and foetus (Griffiths & Campbell, 2015). Drug transfer via the placenta may be either beneficial or detrimental. Beneficial transfer refers to intentional drug administration with the purpose of reaching the foetus via the placenta to exert its effect, for example maternal steroid administration to improve foetal lung development (Griffiths & Campbell, 2015). Detrimental drug transfer may be as a result of a pregnant mother using drugs of abuse or maternal administration of therapeutic drugs, which are harmful to the foetus and not recommended during pregnancy (Griffiths & Campbell, 2015).

1.5.2.2 Drug disposition

Infants are not "miniature adults" and it is very important to keep this concept in mind when considering paediatric post-mortem toxicology (Dolinak, 2013). For this reason the dosage instructions for many medications are different for adults and children under a specific age (Middleberg, 2014). Physical changes that occur in the growing infant and child include an increase in size as well as changes in the body fat ratio (Dolinak, 2013). For example, from birth to adulthood (18 years) there is an approximate five-and-a-half-fold increase in body weight, an approximate three-fold increase in body surface and area and an approximate 16-fold increase in blood volume (Rodman, 1994). These physiological changes can alter the disposition of drugs and their metabolites, and in turn, subsequently alter their detection (and concentrations) in different biological samples following analysis.

The age-dependent increase in blood volume is also important when interpreting toxicological results as this alters the concentration and distribution of drugs in the blood (Rodman, 1994). Physiological changes occurring during the development from infant to adult refer to a developing metabolism and maturation of enzymes responsible for drug metabolism and subsequent elimination. Drug metabolism happens primarily in the liver (hepatic metabolism) (Mahmood, 2016). CYP3A4 is the most abundant cytochrome P450 (CYP) enzyme in the adult liver, but is present in very low levels in the fetal liver and reaches approximately 35 % of adult activity one month postnatally (Lacroix et al., 1997). All processes involved in drug excretion by the kidneys are immature at birth and reach adult values at between two and seven months

of age (West, Smith & Chasis, 1948; Barnett et al., 1949; Arant, 1978; Brown & Campoli-Richards, 1989; Loebstein & Koren, 1998). Drugs may therefore not be appropriately metabolised and eliminated and dosing regimens need to be adjusted accordingly.

1.5.3 Infant pharmacodynamics

Pharmacodynamics refers to the processes resulting in biological effects following the administration of a substance, including substance interaction with receptors and DNA, alteration of gene expression, modification of mediator molecules (e.g. second messengers), cell growth induction and changes in organ function (Heinrich-Hirsch et al., 2001; Middleberg, 2014; Lappas & Lappas, 2016). It must be considered that due to differences in development, physiological effects of drugs may therefore also differ between adults and infants (Umweltbundesamt, 2004).

There is a current lack of information about the effect that developmental changes of the human body have on specific drug pharmacodynamics. This poses a big issue as this directly affects drug dosing guidelines: if the effects of ontogeny on specific drug pharmacodynamics are unknown, it is impossible to have optimal drug dosing guidelines in the infant population (Dolinak, 2013). This may result in prescribed drug dosing regimens, which may cause toxic and adverse effects in this population and which may ultimately lead to death. Likewise, there is still a limited understanding of the specific effects that certain drug exposure may have on infant and child development, as well as effects elicited from drugs of abuse. One of the challenges with infant drug dosing is that this population is largely excluded from clinical trials, which limits our understanding of pharmacokinetics and pharmacodynamics of even common therapeutic drugs, such as antimicrobials and antivirals in infants. Our understanding of recreational drug exposure and the effects in infants is even further limited as we cannot investigate these drugs in infant populations (Roberts et al., 2014).

1.6 Medico-legal implications

Multiple issues may present themselves with the interpretation of post-mortem toxicological results in infants. One reason for this are the differences between infant and adult pharmacodynamics and pharmacokinetics. The mere presence of a substance does not mean that it caused or even contributed to the death of an infant, but it is important to consider all

possibilities, especially due to the vulnerability of infants. Other than infant vulnerability, infant neglect and abuse are other important factors to consider when drugs are detected due to intentional or unintentional exposure. To prevent incorrect classification of a case as SIDS, toxicological data illustrating exposure, is key (Bajanowski et al., 2005).

To date, few studies investigating the post-mortem presence of drugs in infants have been reported. Table 1.1 represents a summary of some of the published studies reviewing the role of toxicological testing in infants. Studies included in Table 1.1 were not selected based on strict inclusion/exclusion criteria: recent studies (studies published after 2000) were included. Studies with relevant findings were selected, i.e. studies that reported on the detection of drugs in infants, whether forensic or clinical. Not only are there very few published post-mortem toxicological studies, but there are none in Africa (to the authors' knowledge). Table 1.1 highlights the role that toxicological testing may play in a more reliable and holistic determination of the cause of death, the importance of considering detection of OTC medication as possible contributors to death, as well as the importance of investigation of circumstances surrounding death in cases where drugs are detected that should not be present in infants, for example methadone.

Table 1.1: *Key published studies that have reviewed or reported on toxicological testing in infant deaths.*

Authors	Year	Country	Study Type	Sample size	Toxicology results	Key findings
Campbell & Collins	2001	USA	Retrospective (ten years)	709 paediatric cases	Cause of death in 11 cases were as a result of drug exposure.	Toxicological testing should be done in all paediatric cases and not only in cases with suspicion of poisoning or overdose.
Langlois et al.	2002	Australia	Retrospective (five years)	117 SIDS cases	Positive for drugs in 16 % (19 cases).	Cause of death changed in three of the cases following toxicology. Without toxicology testing the possible drug contribution to death would have been unknown.
Baker et al.	2003	USA	Case report	Five cases	All positive for diphenhydramine Cause of death changed in all 5 cases from SIDS (2 cases) or unknown (3 cases) to drug-related.	Toxicological testing should be done in all infant cases. Without toxicology testing, the cause of death in all these cases would have incorrectly been classified as SIDS or unknown.
Bajanowski et al.	2005	Germany	Retrospective (three years)	292 SIDS cases initially thought to be natural deaths	Toxicological testing identified poisoning in two cases (0.7 %).	Toxicology testing is important in all infant cases to prevent inaccurately classifying a death as SIDS.
Rimsza & Newberry	2008	USA	Retrospective (one year)	90 unexpected infant deaths (tox testing done in 21)	48 % cases positive for OTC CCMs 50 % multiple OTC CCMs exposure One case: OTC CCMs were cause of death.	Cannot ignore OTC CCMs as a contributor to infant death. Their detection should raise suspicion. Recommended revision of instructions on medicine labels.
Weber et al.	2008	UK	Retrospective (ten years)	546 SUDI cases	Toxicology was only done in selected cases. Cause of death remained unexplained in two thirds of the cases.	Toxicology is a very important ancillary test and if it became routine it may reduce the number of infant deaths without an established cause of death.
Mistry et al.	2010	UK	Case report	Two cases	Methadone detected in two infants. Methadone should only be present in infants with mothers on methadone maintenance programs.	Lack of peer-reviewed published infant forensic toxicological data which is necessary for accurate interpretations. Interpretations rely mainly on published adult data.
Paul, Simms & Mahesan	2017	USA	Case report	Three cases	Methadone was detected in all three cases.	Methadone should not be present in infants (its presence may point to either neglect or lack of awareness in case of a caregiver). Lack of peer reviewed published infant toxicological data makes accurate interpretation difficult.

(OTC: over-the-counter; CCM: cough and cold medication)

The IMRs in developing countries are higher than in developed countries and it is indeed in these developing countries where these studies and collection of data is urgently needed. The lack of peer-reviewed publications on infant post-mortem toxicological data often results in having to rely on adult toxicological data to interpret infant toxicological results. This is problematic given the variations between adult and infant pharmacokinetics and pharmacodynamics as discussed, which may thus result in drawing inaccurate conclusions from the results (Mistry et al., 2010; Paul, Simms & Mahesan, 2017).

All of the reviewed studies highlighted the importance of toxicological testing as an ancillary investigation in all SUDI cases and not only in the ones where there was a suspicion of poisoning or overdose. The reason for this is that in the absence of toxicological testing, the possible contribution of drugs to death might have gone undetected (Campbell & Collins, 2001; Langlois et al., 2002; Weber et al., 2008). Emphasis was placed on the importance of toxicological analyses in preventing incorrect classifications of SIDS deaths, when exposure to drugs may have played a contributory role in death (Baker et al., 2003; Bajanowski et al., 2005).

1.7 Knowledge gap and motivation

The third SDG is aimed at reducing under-five child mortality by 2030 (United Nations, 2015). Given the risk of intentional and unintentional drug exposure causing or contributing to infant death, toxicological analyses may provide value in understanding infant mortality. However, little is known about the prevalence of drugs in SUDI cases at SRM, since biological samples are not routinely collected for toxicological analyses at autopsy.

While research is limited, previous studies have highlighted the importance of toxicological investigations in infant deaths, not only in those cases where drug use or administration is apparent or where there are suspicions of poisoning and/or exposure (Table 1.1). For example, Baker et al. (2003) determined that without toxicological testing, drugs would not have been detected in SUDI cases, and the presence and possible contribution of diphenhydramine (an OTC antihistamine) to death in the case under review in the paper would have remained unknown (Baker et al., 2003).

Performing toxicological testing in SUDI cases may result in detection of a variety of drugs, some of which may not necessarily have contributed to the death of the infant. However,

detecting these drugs may provide valuable information regarding the case circumstances, such as inappropriate exposure or administration of drugs to which an infant should not have been exposed (whether intentionally or unintentionally). If drugs of abuse or medication not recommended for infant use are detected in these cases, it may be important to investigate the household circumstances in which the infant was living. In some instances, this may result in other children in the household being removed from the care of the parents and in some cases charges of child abuse or child neglect may be opened.

Given the current lack of published data both nationally and globally (Arnestad, 2013; Paul, Simms & Mahesan, 2017), additional research is needed to establish what types of drugs are detected in SUDI cases and what sample types are of interpretative value. Detecting drugs in local SUDI cases that would not typically have received toxicological analyses, would strongly motivate for the implementation of routine toxicological investigations. This would theoretically change the way in which SUDI cases are investigated in the future because if drugs are detected in these infants, it may result in legal repercussions for parents and/or caregivers because of child abuse and/or child neglect.

1.8 Aims and objectives

The aim of this study was to determine the presence of drugs in a pilot cohort of SUDI cases at SRM, in order to demonstrate a proof of concept of the value of toxicological testing in these cases and to motivate for routine infant toxicological testing.

This aim was achieved by the following objectives:

- Recruitment of a pilot cohort of SUDI cases admitted to SRM and subsequent collection of several biological samples from the infants.
- Assessment of the presence of drugs in different sample types.
- Investigation and evaluation of the types of drugs in the cohort and performing a descriptive analysis on the findings.
- Assessment of the association between the presence of drugs and:
    - self-reported maternal behaviour (e.g. breastfeeding and self-reported drug use),
    - cause of death (e.g. infectious death and drugs detected).

It was hypothesised that there will be drugs detected in a deceased infant sample population, particularly because they are at higher risk to unintentional drug exposure.

Chapter 2: Methods

2.1 Study design

This study was a cross-sectional study with a quantitative research paradigm and included prospective sample and data collection and analysis. The study population consisted of a random group of SUDI cases admitted to SRM.

2.2 Participants

The cohort in this pilot study consisted of 30 SUDI cases admitted to SRM between 28 May 2019 and 17 October 2019. The age of study participants ranged from three days to seven months, with an average age of 2 months and 26 days (median age: 2 months, 15 days). Of the study participants, 16 (53 %) were female and 14 (47 %) were male. The University of Cape Town (UCT) Department Research Committee and Human Research Ethics Committee (HREC) approved this study (REF: 445/2015) (Appendix A.1).

2.2.1 Case identification and recruitment

Cases potentially included in this study were identified by a daily review of the autopsy allocation list of SRM (see inclusion and exclusion criteria below). After admission of the body to SRM, the next-of-kin had to identify the deceased, where after an autopsy was performed by the authorised medical practitioner. In all included cases, identification took place prior to the medical examination. The researcher thus requested informed consent from next-of-kin of the deceased when they came to SRM for the identification process. The informed consent process was conducted in a private room at SRM commensurate with published guidelines (Heathfield et al., 2017) (Appendices B.1 and B.2).

2.2.2 Inclusion and exclusion criteria

Cases were included in this study if they were admitted to SRM as suspected SUDI cases. The deceased individual had to have been born alive and had to be under the age of one year (as

identified by the birth certificate) at the time of death declaration. Cases were only included if informed consent was obtained from the next-of-kin of the deceased.

Cases were excluded if infants were stillborn if the deceased was hospitalised for a period of more than 24 hours before death or if the body of the deceased was severely decomposed or burnt. These cases were excluded since the various conditions may alter the reliability of subsequent post-mortem drug detection in different biological samples of the deceased individual.

2.2.3 Clinical and social history

Clinical and social history pertaining to the 30 SUDI cases was obtained from the FPS006(b) form, which was filled in by the next-of-kin when they came to SRM for identification of the deceased. This included whether the infant was ill prior to death, whether the infant received medical attention (doctor, clinic, pharmacy or traditional healer), whether any medication was given to the deceased prior to death, whether the mother uses drugs and whether the infant was breastfed or not. The cause of death, type of death and alleged manner of death, as determined by the medical practitioner, were recorded from the post-mortem report (Appendix C.1).

2.3 Sample collection

Blood, urine, vitreous humour and hair were selected as sample types for collection and analysis based on recommendations by The International Association of Forensic Toxicologists (Committee of Systematic Toxicological Analysis, 2018). All four sample types were recommended for collection in the post-mortem setting. Blood was selected since it has been described as the most important post-mortem specimen for analysis: blood provides insight into more recent exposure to a drug (such as one day). Urine was selected since drugs are often present in higher concentrations which will facilitate the detected thereof and urine can provide insight into drug exposure ranging from days to weeks (depending on the type of drug) (Committee of Systematic Toxicological Analysis, 2018). Vitreous humour is a valuable post-mortem sample type due to its anatomically protective location which makes it more resistant to degradation (compared to urine and blood). Vitreous humour provides insight into a short window of exposure and was a valuable specimen in this study since blood was not always available for collection in infants. Hair is easy to collect (non-invasive) and its analysis

provides one with a longer history of exposure (weeks to months) (Committee of Systematic Toxicological Analysis, 2018).

The analysis of blood and vitreous humour provide more value when quantitative data are associated with the drugs detected, however, for the purpose of this study the detection of drugs in these two sample types sufficed to suggest short-term drug exposure (especially if detected in both).

Samples were collected at the autopsy by the authorised medical practitioner at SRM. Blood, urine, vitreous humour and hair were collected from each infant, where available. The collection site used for sample collection was at the discretion of the medical practitioner and was largely dependent on the type of autopsy conducted (full, partial or external) (Table 2.1). During a full autopsy, the body was opened completely, whereas during a partial autopsy the body was opened partially. During an external examination, the body was not opened. Blood and vitreous humour were collected in grey-top vacutainer tubes containing sodium fluoride (preservative) and potassium oxalate (anticoagulant). Urine was collected in 15 mL sterile conical centrifuge tubes without any preservative. A bunch of hair with a diameter of approximately 7mm was collected from the posterior vertex region and was placed inside a paper fold, which was placed inside an envelope (Table 2.1).

Table 2.1: *Collection sites and techniques used for collection of biological samples.*

Sample	**Collection technique / equipment used**	**Collection site(s)**
Blood	Insert needle with syringe into heart and draw blood	Left and right sides of heart
	Insert needle with syringe into cardiac region and draw blood	Blind-stick collection in cardiac region
Urine	Insert needle with syringe into bladder and draw urine	Bladder
	Insert needle with syringe into lesser pelvis region and draw urine	Blind-stick collection in lesser pelvis region
Vitreous humour	Insert needle with syringe into eye and draw vitreous humour	Left and right eyes

Hair	Pulled hair out of scalp	Back of head (posterior vertex region)
	Cut hair close to scalp with scissors (sterilised with ethanol)	

While femoral blood is the preferred sample from a toxicological point of view, as it is less prone to post-mortem artefacts (Pounder & Jones, 1990); cardiac (heart) blood was collected due to the limited volume of blood available in the infants. This is a sample that is also suitable for screening purposes. Blind-stick collection was performed in cases where the body was not opened. For the purpose of this study it was an adequate collection technique since the collected sample only underwent a drug screen and not quantitative analysis.

The preferred collection site for hair was the vertex posterior region as this was the collection site recommended by published guidelines (Kintz, 2019). This region typically has less growth variability compared to the rest of the head and is affected to a lesser degree by age- and sex-related influences (Kintz, Villain & Cirimele, 2006).

Blood, vitreous humour and urine were kept on ice from collection until transfer to the laboratory. At the laboratory, these samples were refrigerated at 4 °C until analysis. Hair samples were transported and stored at room temperature. The process of transporting the samples was compliant with UN guidelines (United Nations, 2011). A unique identifying number was given to each case and each sample within a case and was used throughout the study to maintain confidentiality.

2.4 Qualitative toxicological analysis

2.4.1 Chemical and reagent preparation

The internal standard (IS) stock solution contained doxepin-d3 and diazepam-d5 (10 µg/mL) in methanol and was obtained from Restek (Restek Corporation, Bellefonte, PA, USA). All chemicals used were of high-performance liquid chromatography (HPLC) grade or higher. Acetonitrile (ACN) was obtained from Romil Pure Chemistry (Romil Limited, Waterbeach, CAM, UK). Dichloromethane (DCM) was obtained from Alfa Aesar by Thermo Fisher Scientific (ThermoFisher (Kandel) GmbH, Kandel, Germany). Ethanol (Ethyl alcohol, pure) was obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Methanol (Methanol

215) was obtained from Romil Pure Chemistry (Romil Limited, Waterbeach, CAM, UK). Hydrochloric acid (HCl) (standardised solution, 5.0 N) was obtained from Alfa Aesar by Thermo Fisher Scientific (ThermoFisher (Kandel) GmbH, Kandel, Germany). Deionised water was prepared with a Milli-Q® Integral Water Purification System for Ultrapure Water (Merck, Burlington, MA, USA).

2.4.2 Sample preparation

2.4.2.1 Blood and vitreous humour

To an aliquoted volume of 100 µL of blood or vitreous humour, 600 µL ACN and 4 µL IS were added in a 1.5 mL micro-centrifuge tube. The tube was vortexed for one minute after which it was centrifuged (five minutes at 13 000 rpm) and 600 µL of supernatant was transferred to a borosilicate glass tube. The micro-centrifuge tube with the pellet was discarded. The supernatant in the borosilicate tube was evaporated using an XcelVap® automated evaporation instrument (Horizon Technology, Inc., Uppsala, Sweden). The residue was reconstituted in 200 µL of deionised water and following vortexing, all 200 µL was pipetted into a glass insert within a 2 mL screw neck glass vial. The vial was vortexed for one minute before placing it into the instrument autosampler for injection.

2.4.2.2 Urine

To a volume of 200 µL of urine, 100 µL ACN and 8 µL IS were added in a 1.5 mL micro-centrifuge tube. The tube was vortexed for one minute followed by centrifugation (five minutes at 13 000 rpm) and 200 µL of supernatant was added to a 2 mL screw neck glass vial. Deionised water (800 µL) was added to the vial, after which it was vortexed for one minute before placing it into the instrument autosampler for injection.

2.4.2.3 Hair

Sample preparation for hair analysis consisted of three steps; washing, pulverisation and dry down and reconstitution.

The washing step started with weighing 20 mg hair out in an Omni Bead Ruptor Bead Mill tube (Omni, Inc., Kennesaw GA, USA). The hair was pushed to the bottom of the tube using a clean rod and 1 mL ethanol was added. The tube was vortexed for 10 seconds and left at room

temperature for 10 minutes with a closed lid. The liquid was discarded, and 1 mL DCM was added. The tube was vortexed for 10 seconds and then left at room temperature for 10 minutes with a closed lid. The liquid was again discarded, and the process repeated. Following this, 800 µL of supernatant was transferred to a 1.5 mL micro-centrifuge tube (the 'wash supernatant').

An extraction solution was prepared that consisted of a methanol and IS mixture (IS concentration = 200 ng/mL) and aqueous HCl (1 M) in a 4:1 ratio. To pulverise the hair, four metal beads (specialised for pulverisation) and 1 mL of extraction solution were added to the Omni Bead Ruptor Bead Mill tube containing washed hair. The tube was left at room temperature for 10 minutes with a closed lid after which it was loaded onto an Omni International Bead Ruptor Elite Bead Mill Homogenizer (Omni, Inc., Kennesaw GA, USA). This was run at 5.5 m/s for one minute, with a dwell time of 30 seconds for five cycles. The tube was centrifuged for five minutes at 4 000 rpm and 800 µL of supernatant was transferred to a 1.5 mL micro-centrifuge tube.

This supernatant as well as the 'wash supernatant' were centrifuged for five minutes at 13 000 rpm. A volume of 700 µL of supernatant of each were transferred separately to two different glass borosilicate tubes. The supernatant in the glass borosilicate tubes was evaporated using an XcelVap® automated evaporation instrument (Horizon Technology, Inc., Uppsala, Sweden). The residues were reconstituted in 150 µL deionised water and following vortexing, all 150 µL from each tube was pipetted into a glass insert in separate 2 mL screw neck glass vials. The vials were vortexed for one minute before placing them into the instrument autosampler for injection.

In four cases, the collected amount of hair was limited and only 5 mg was available as opposed to the required 20 mg. Therefore, the sample preparation procedure was adjusted, whereby all the reagent volumes were reduced to 25 % of the original volume as per the procedure described above.

2.4.3 High-resolution LC-QTOF-MS screening

Prepared blood, vitreous humour and hair samples underwent toxicological screening using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF) analysis with a SCIEX X500R Q-TOF (quadrupole time-of-flight) System (MDS Analytical Technologies (US) Inc., Framingham, MA, USA). Samples were analysed using the SCIEX vMethod™ Application for Forensics Toxicology (Fu et al., 2017).

This method is commonly used in forensic toxicology to assist in determining and/or detecting unknown compounds in complex sample matrices (Fu et al., 2017). This method has the ability to instantly switch between MS and MS/MS scans which enable easier identification of compounds because detailed structural information is more easily acquired (Fu et al., 2017).

Chromatographic separation was performed on an ExionLC™ AC HPLC system. A Phenomenex Kinetex® 2.6 µm phenyl-hexyl column (50 mm x 4.6 mm) was used for chromatographic separation of analytes. Mobile phase A (MP-A) was 10 mM ammonium formate in water and mobile phase B (MP-B) was 0.05 % formic acid in methanol. A linear gradient with MP-B was run at 600 µL/min from 10 % to 98 % for seven minutes followed by 90 seconds of 98 % MP-B and finally 10 % MP-B for one minute (Fu et al., 2017).

Analysis was performed in positive ionisation mode. MS and MS/MS data were collected using SWATH® Acquisition. Data was processed and analysed using the SCIEX OS Software (AB Sciex Pte. Ltd., Woodlands, Singapore). SWATH® Acquisition is a data independent acquisition technique which means it allows for the all-inclusive detection of almost every detectable compound in a sample (Sciex, 2020). The generated data are processed against a library of 750 drugs which introduce an element of directed processing. It is possible that ions for a specific compound were collected but not processed appropriately against the library spectra, which may result in the compound not being reported out. A benefit of high-resolution mass spectrometry and SWATH analysis is that the analyst can re-process the data to look for a compound not originally in the library (Sciex, 2020).

2.5 Data analysis

2.5.1 Toxicological data processing

Targeted data processing was performed using SCIEX OS Software. This data processing method was set up prior to this study in the Clinical Pharmacology Laboratory (Groote Schuur Hospital). Confidence criteria used to distinguish positive findings included mass error, retention time (RT), isotope ratio difference and library score (Fu et al., 2017). Data processing was conducted by technicians within the Clinical Pharmacology Laboratory (Appendix C.2).

Drugs were considered as present if the mass error, RT, isotope ratio difference and library score passed and if both the ISs were detected. These elements were set by the technicians

based on their routine toxicology processing method, which was automated on the instrument. The instrument software was designed to determine pass/fail based on technical components. If any of the mass error, RT, isotope ratio difference or library scores did not pass or if only one or none of the ISs were detected, the drug was not considered present (detected) and it was considered as a "failed" analysis (the IS in these cases failed).

2.5.2 Statistical analyses

Basic descriptive statistics with simple summaries were performed on the recorded data. Five-number summaries were performed on the number of drugs detected per sample type. The average number of samples collected per case was calculated. Percentages of cases where the infant was ill prior to death, where the infant received medical care, where drugs were administered prior to death, where the infant was breastfed and where the mother used drugs of abuse, were calculated. A one-way ANOVA was performed to test for a significant difference between the number of drugs detected within the four different sample types.

Fisher's Exact Tests were performed to determine whether there were significant differences between the following: the presence of drugs in bodily fluids (blood, vitreous humour and hair) and self-reported breastfeeding, as well as the presence of drugs in hair and self-reported breastfeeding. Fischer's Exact Tests were performed to test whether there were significant differences between infection as cause of death (determined by the medical practitioner) and the presence of drugs of abuse, OTC drugs (for symptom relief) and antibiotics respectively. A p-value of 0.05 was used to evaluate statistical significance. Statistical analysis was performed with GraphPad Prism v8.3.0.

Chapter 3: Results

3.1 Sample collection

The number of cases for which the four different sample types were collected and prepared for toxicological analysis is shown in Figure 3.1. Blood was the most abundant sample and urine the least abundant sample. At least one sample was collected and analysed per case. An average of 2.5 samples were collected per case, and in total, 75 samples were collected for the combined 30 cases. Sample analysis was not successful for every sample collected and therefore results were not available for every collected sample.

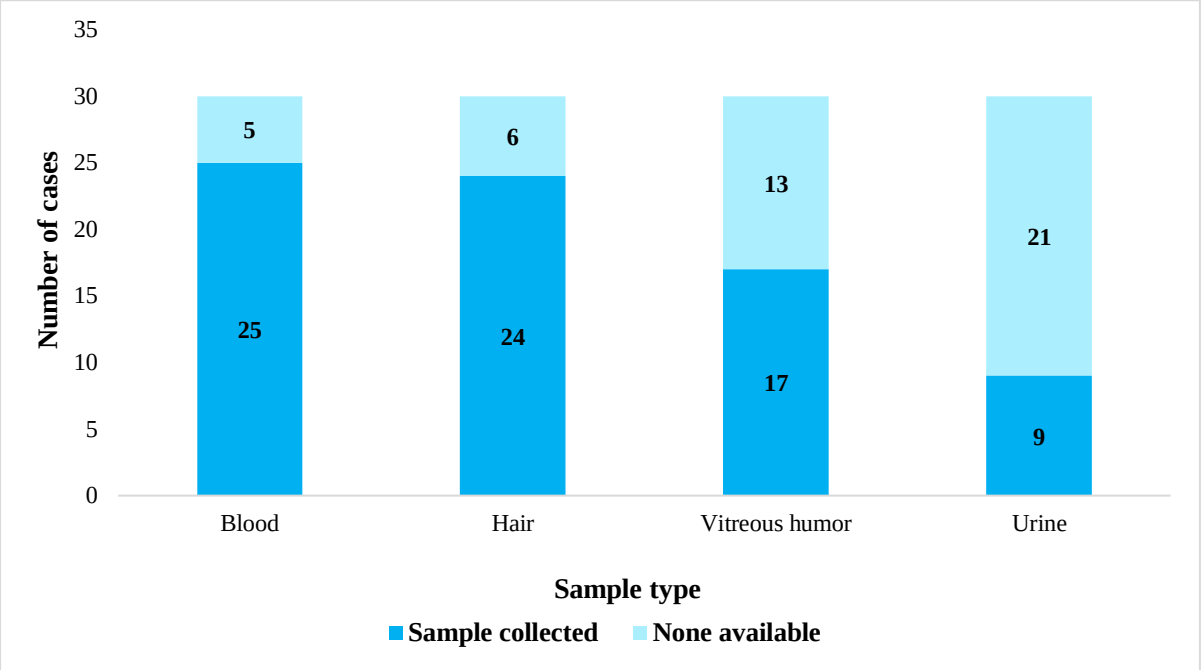

Figure 3.1: *Number of cases for which each sample type (blood, hair, vitreous humour and urine) was collected and prepared (n = 30 cases).*

3.2 High-resolution LC-QTOF-MS screening results

3.2.1 Presence of drugs in various sample types

Results were deemed acceptable according to confidence criteria (section 2.5.1) for 28 of 30 cases. The remaining two cases (011 and 012) only contained hair samples of low mass, for which the internal standard (IS) did not pass (section 2.5.1). These samples could not be reanalysed due to limited sample. The internal standard (IS) passed during toxicological analysis for at least one of the available samples per case in 93 % of cases (n = 28). In two of these cases (excluding the two mentioned previously where hair was the only sample – 011 and 012), namely cases 023 and 026, the IS failed in the hair analysis, however other samples were also available for these cases, which passed all quality criteria during toxicological screening. The adjustment of the sample preparation and the limited hair sample available may have contributed to the failure of the IS in these four samples. Of the accepted cases, drugs were detected in 89 % (n = 25/28) (Figure 3.2) with more than one drug detected in 80 % of these cases (n = 20/25). Overall, drugs were detected in 49 of the 75 collected samples.

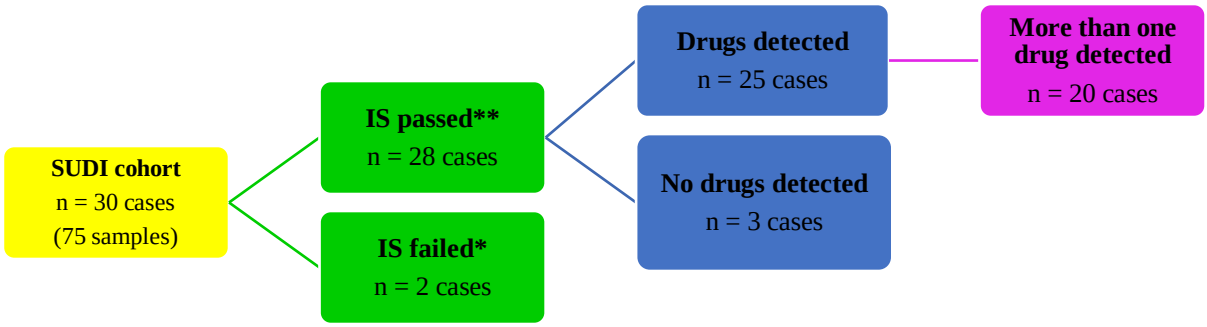

Figure 3.2: *Flow diagram indicating the number of cases in which drugs were detected. Of 75 total samples collected in the 30 cases, drugs were detected in a total of 49 samples, no drugs were detected in 22 samples, and in four samples the IS failed and reanalysis could not be performed (IS: internal standard; *In two cases, only hair was present and the IS failed (cases 011 and 012). These samples could not be reanalysed. **In two cases (cases 023 and 026), the IS failed, however, other specimens were present and provided valid results for discussion).*

The number of drugs detected in each sample type per case was counted, and the ranges for each sample type are depicted in Figure 3.3. Hair had the highest range, with between one and six drugs detected per sample. A one-way ANOVA determined that there was a significant

difference between the number of drugs detected in the four different sample types ($p < 0.0001$) (Figure 3.3). Further, hair was the only sample type in which at least one drug was always detected. For some cases, no drugs were detected in the other three sample types when hair was positive, highlighting the importance of hair as a sample type in the investigation of SUDI cases.

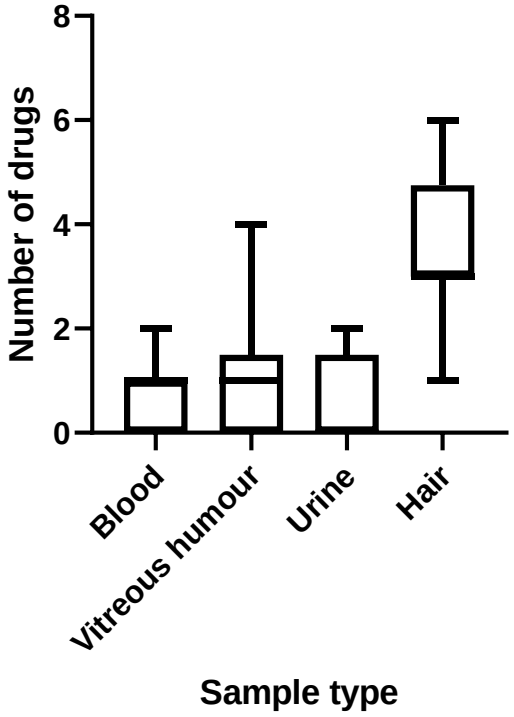

Figure 3.3: *Box and whisker plot for the number of drugs detected in the four different sample types (ranges blood:0 - 2; vitreous humour: 0 - 4; urine: 0 - 2; hair: 1 - 6).*

3.2.2 Types of drugs detected during toxicological analysis

The detected drugs were divided into three different categories: drugs of abuse (red), drugs permissible for infant use (green), drugs not recommended for infant use (blue) (Figure 3.4). The number of cases for which these drugs, or combinations of the drugs, were detected can be seen in Figure 3.4.

In many cases (n = 10/28), a combination of drugs permissible for infant use and drugs not recommended for infant use was detected (Figure 3.4). In 21 % (n = 6/28) of cases, a combination of drugs from all three different categories were detected.

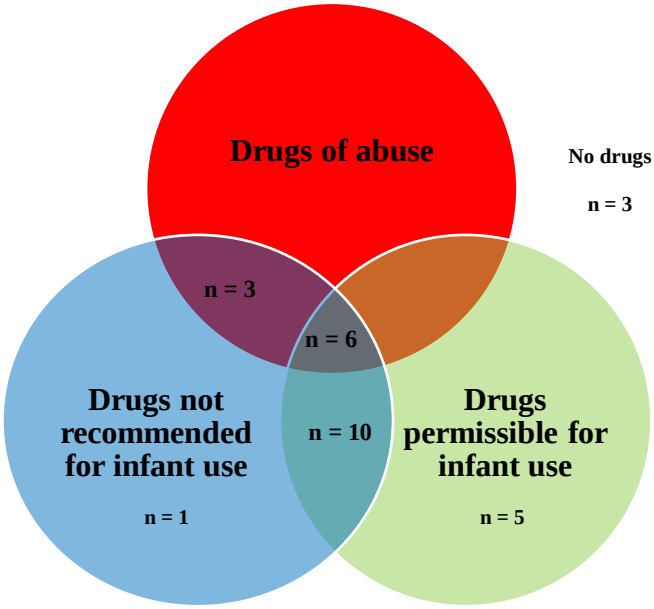

Figure 3.4: *Venn diagram indicating the number of cases in which drugs within the three categories were detected (n = 28).*

A detailed summary of all the detected drugs, including their therapeutic uses, their potential to be secreted into breast milk and the sample type(s) they were found in, can be seen in Table 3.1. The absence of drugs in the analysed samples are indicated as well as the samples in which the IS failed. The drugs for which potential for secretion into breast milk were indicated, were drugs for which published evidence were available.

An example of the visual output of the results of the high-resolution LC-QTOF-MS screening can be seen in Appendix C.3. The example is for the blood sample of case 009 (Appendix C.3) and illustrates the presence of acetaminophen and the two ISs. The chromatogram and analyte retention times, together with the MS and MS/MS fragmentation spectra (and the library matches) are illustrated.

Table 3.1: *Drugs detected in the four samples for the 30 cases and intended therapeutic uses (pink: blood; purple: vitreous humour; yellow: urine; green: hair; grey: breastfed;* *******drug reported to be secreted into breast milk; #amphetamine (metabolite) detected; E: IS error; specimen is not present for a case: not collected for the specific case).*

Detected drugs (*and drug class*)	**Blood** / **Urine** / **Vitreous humour** / **Hair**	**Intended therapeutic use of drug**	**SUDI ID number** 01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
	Acetaminophen *(analgesic)**	Pain relief (FDA, 2016).	
	Caffeine (s*timulant)********	Headache and weight loss medication; short-term treatment of neonatal apnoea (Baselt, 2009a).	
	Sulfamethoxazole *(antibiotic)********	Infections (GIT, respiratory tract, urinary tract) (Smilack, 1999).	
	Verapamil *(calcium channel blocker)********	Angina, hypertension (Rossiter, 2017).	
	Methaqualone *(sedative)*	Drug of abuse (Rossiter, 2017).	
	Methamphetamine *(stimulant)********	Drug of abuse (Sabrini et al., 2019).	
	Tramadol *(opioid analgesic)********	Pain (Baselt, 2009b).	
	Lidocaine *(anaesthetic)**	Regional anaesthetic (Rossiter, 2017).	
	Metoclopramide *(prokinetic agent)**	Nausea and vomiting (Rossiter, 2017).	
	Ondansetron *(opioid analgesic)*	Nausea and vomiting (Cheng, 2011).	
	Chlorpheniramine *(antihistamine)*	Allergic symptoms (Simons, Luciuk & Simons, 1982).	
	Ciprofloxacin *(antibiotic)********	Infections (Rossiter, 2017).	
	Guaifenesin *(expectorant)*	Coughing (Rossiter, 2017).	
	Hydrocortisone *(glucocorticoid)**	Allergies, inflammation (Rossiter, 2017).	
	Quinine (*antimalarial)********	Malaria treatment (Rossiter, 2017).	
	Thiabendazole *(anthelmintic)*	Worm infections (Drugs.com, 2019).	
	None detected		E E E E

In total, 16 different drugs were detected in the 28 cases (Figure 3.5). In 14 % of cases (n = 4/28) multiple drugs were detected among the sample types (blood, vitreous humour and hair). In 64 % of cases (n = 18/28) multiple drugs were detected in hair samples alone (Table 3.1; Appendix C.2). Acetaminophen was the most abundant drug and was detected in 61 % of cases (n = 17/28), and in most of these, (71 %; n = 12/17), it was detected in hair samples. Methaqualone was detected in nine cases (30 %) and in all instances, it was only detected in hair samples. In two of the three cases where methamphetamine was detected, it was also detected in hair samples.

The value of hair as a sample type in detecting drugs was also seen with sulfamethoxazole, where it was found in hair samples in 91 % of cases (n = 10/11) where it was detected (Table 3.1). In all 15 cases where caffeine was detected, it was detected in hair samples. Lidocaine (two cases), quinine (two cases), thiabendazole (one case), tramadol (three cases) and verapamil (12 cases) were all only detected in hair samples.

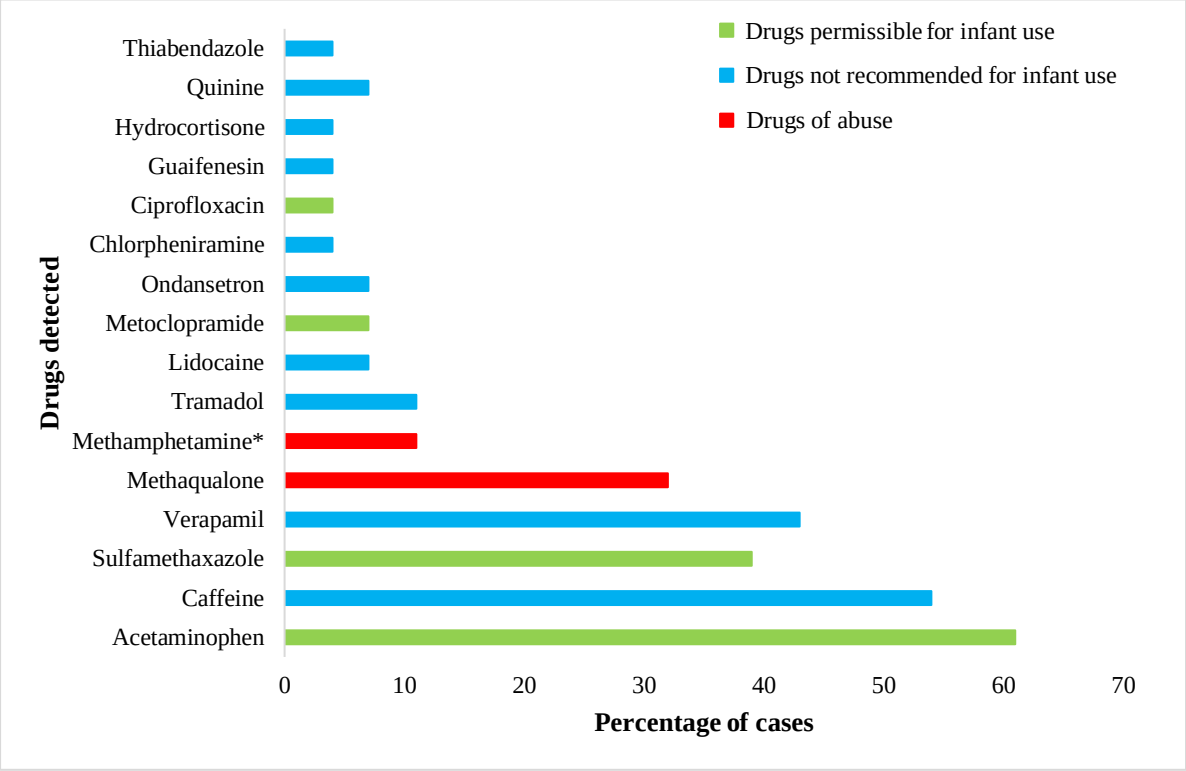

Figure 3.5: *Different drugs detected (n = 28) from toxicological LC-QTOF screening (*in one case where methamphetamine was detected, its metabolite (amphetamine) was also detected).*

3.3 Self-reported maternal behaviour

Figure 3.6 shows the degree to which the next-of-kin self-reported the following information: administration of drugs to the infant prior to death, whether the infant was breastfed or not, whether the mother used drugs of abuse and whether the infant was ill prior to death. The integration of the toxicology results that pertain to each of these categories are given below.

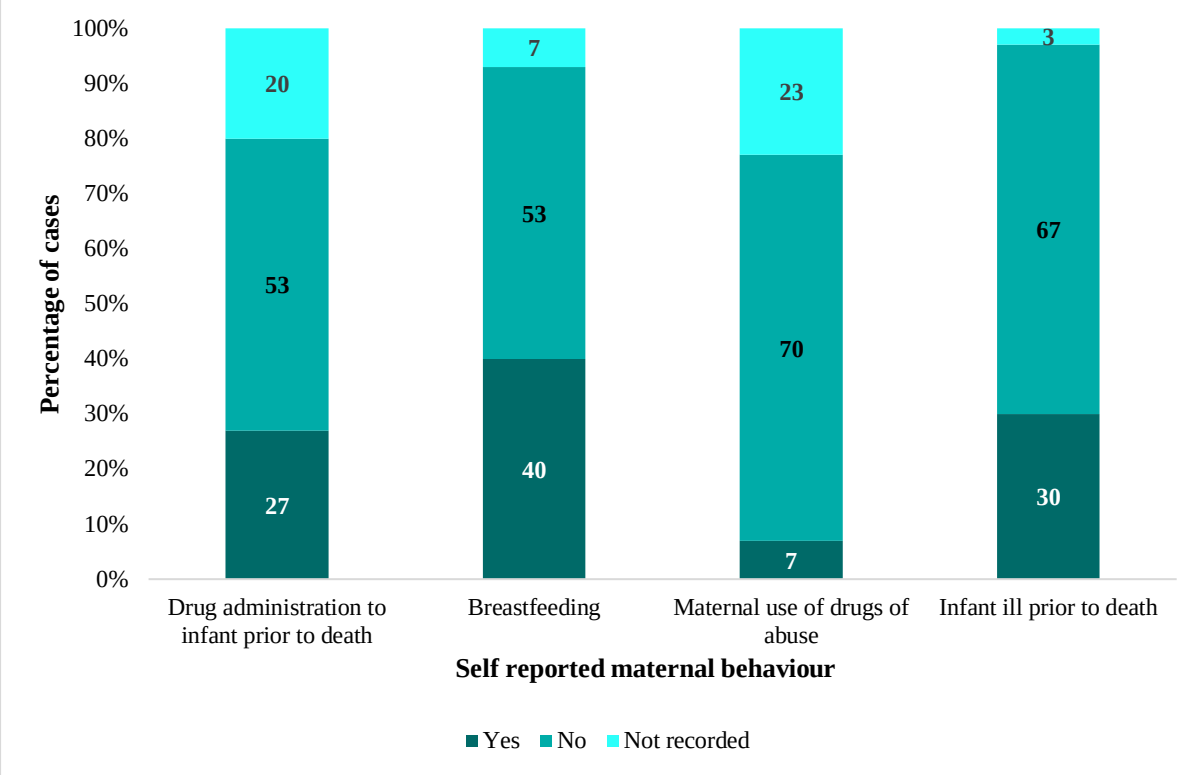

Figure 3.6: *Percentage of cases for which the following self-reported maternal behaviour was recorded: whether drugs were administered to the infant prior to death, whether the infant was breastfed or not, whether the mother used drugs of abuse and whether or not the infant was ill prior to death.*

3.3.1 Self-reported drug administration

In 27 % of the cases (n = 8/28) the mother indicated that drugs were administered to the infant prior to death (Figure 3.6). The self-reported administered drugs included paracetamol (acetaminophen), antibiotics, antiretrovirals (ARVs), proton pump inhibitors (PPIs), multivitamins and CCMs (Figure 3.7; Appendix C.1).

Acetaminophen administration to infants prior to death (as reported by the next-of-kin) took place in five cases (cases 011, 014, 015, 022 and 030) (Appendix C.2) and was confirmed in 80 % of the cases (n = 4/5) for which administration was reported by the mother. The IS failed in the 5th sample (case 011) (Appendix C.1) and the presence of acetaminophen could thus not be confirmed, however the presence of acetaminophen in hair would not have supported whether the infant as exposed to acetaminophen acutely.

Antibiotics were reportedly administered to three infants prior to death, as reported by the next-of-kin (Figure 3.7). In two of these cases, no antibiotic was detected and in one case the IS failed (case 011). However, two antibiotics, sulfamethoxazole, and ciprofloxacin, were detected in other cases during toxicological analysis: sulfamethoxazole was detected in 39 % of the cases (n = 11/28) and ciprofloxacin was detected in one case (Figure 3.5). No ARVs, PPIs, CCM or multivitamins were detected during the LC-QTOF targeted screen.

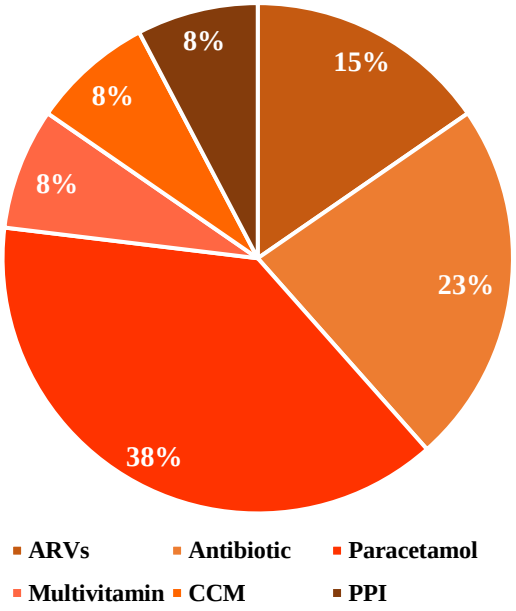

Figure 3.7: *Pie chart indicating the different types of drugs administered to the infants as reported by the next-of-kin (ARVs: antiretrovirals; CCM: cough and cold medication; PPI: proton pump inhibitor).*

3.3.2 Self-reported breastfeeding

Drugs which have been reported to be secreted into breast milk were detected in all 25 cases where drugs were detected (Table 3.1). Of the 30 infants, 12 were reported to have been breastfed (40 %) (Figure 3.6). No drugs were detected in 33 % of cases (n = 4/12) where the

mother reported that the infant was breastfed. For the other eight, the drugs detected included methamphetamine, methaqualone, caffeine, sulfamethoxazole, verapamil, tramadol, metoclopramide, acetaminophen, guaifenesin, hydrocortisone, ondansetron, quinine and lidocaine (Appendices C.1 & C.2).

A Fisher's Exact test determined that there was no significant difference between the presence of drugs in bodily fluids (blood, vitreous humour and urine) and breastfeeding ($p > 0.9999$). There was also no significant difference between the presence of drugs in hair samples and breastfeeding ($p > 0.9999$).

3.3.3 Self-reported maternal use of drugs of abuse

Maternal drug abuse was reported in 7 % of cases (n = 2/30) (Figure 3.6) (cases 006 and 017). It is however unknown if the reported maternal drug use was historical or current. The value of knowledge of historical maternal drug use includes that it may explain the presence of drugs in hair (related to historic use or maternal transfer during pregnancy) versus the presence of drugs in blood, urine and/or vitreous humour (related to inadvertent acute exposure). This hinders the possibility of drawing accurate conclusions between reporting of maternal drug use and detection of drugs of abuse since it is unknown whether the mother was using the drugs during pregnancy and/or thereafter. In both these cases, the drug of abuse was methamphetamine. Methamphetamine was detected in these two infants, as well as in a third infant (11 % of cases; n = 3/28) (Table 3.1). Amphetamine (metabolite of methamphetamine) was also detected in one of the three cases in which methamphetamine was detected (case 017) (Table 3.1). In all three cases where methamphetamine was detected (cases 006, 017 and 021), methaqualone was also detected (Appendix C.2). Methaqualone was only detected in hair samples and was detected in 32 % of cases (n = 9/28).

3.3.4 Self-reported infant health status

The health status of the infant prior to death (n = 30), as reported by the next-of-kin, can be seen in Figure 3.6. The cause of death as determined by the medical practitioner is shown in Figure 3.8.

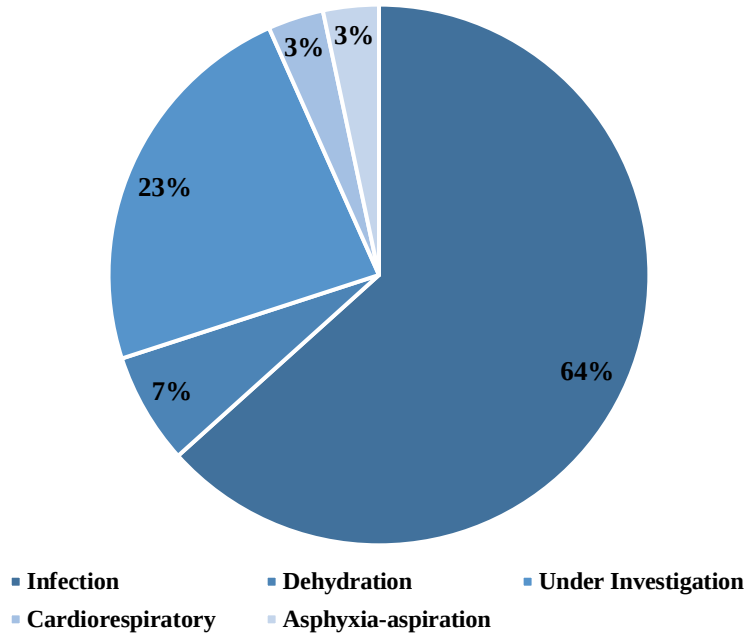

Figure 3.8: *Pie chart indicating the pathological cause of death as determined by the medical practitioner following autopsy (n = 30).*

In 30 % of cases (n = 9/30) the mother reported that the infant was ill prior to death (Figure 3.6). In 67 % of these cases (n = 6/9) the infant received medical care (Appendix C.1). However, infection was the most common cause of death in the study population, as determined by the medical practitioner (64 % of cases; n = 19/30). In 74 % of the cases (n = 14/19) where infection was the cause of death, it was respiratory infection (Appendix C.1). Therefore, there was a misalignment between the mother's recognition of illness and the infectious cause of death established by the medical practitioner.

While the toxicological findings showed a high rate of detection of antibiotics or acetaminophen in the infants, Fischer's Exact Tests determined that there was no significant difference between infection as the cause of death and the presence of drugs of abuse, antibiotics or acetaminophen (OTC medication used for symptom relief) respectively.

Chapter 4: Discussion and conclusion

The aim of this study was to determine the presence of drugs in a pilot cohort of SUDI cases at SRM, in order to demonstrate a proof of concept of the value of toxicology in these cases and to motivate for routine toxicological testing. This study was the first study of its kind to be performed at SRM in terms of prospective collection of samples for toxicological analysis in infants. To this end, a pilot cohort of 30 SUDI cases were included in this study and over 750 drugs were screened for in 75 samples, representing blood, urine, vitreous humour, and hair samples. A total of 16 different drugs were detected in 49 different samples in 25 cases. In nine of these cases, drugs of abuse were detected (Figure 3.5; Table 3.1). If toxicological testing were not performed in these cases, the presence of these drugs would have gone unnoticed.

4.1 Detected drugs of abuse

Methamphetamine and methaqualone (which together with diphenhydramine is known as ‘mandrax’) were the two drugs of abuse that were detected in the study population. Methaqualone was detected in nine cases and in three of those cases, methamphetamine was also detected (Figure 3.5; Table 3.1). A case report published in 1976 reported the intentional administration of methaqualone to a seven-year old male child, following analysis of urine and blood samples. This resulted in removing the child out of the mother’s care (Rogers et al., 1976). To the author’s knowledge, there are no publications concerning the detection of methaqualone in infants.

In 2019, a mother in Louisiana, USA, was accused of murdering her six-week-old daughter by breastfeeding her while she herself used methamphetamine (Vasudevan, 2019). Toxicological testing of maternal and infant blood samples produced positive methamphetamine results and the cause of death was determined to be methamphetamine toxicity (Vasudevan, 2019). In this case, the cause of death was not obvious upon arrival. Without toxicological testing and reporting of maternal drug use, the case would have not necessarily been so easily resolved and the necessary steps to ensure the safety of her other children might not have been taken.

There is a lack of published data on both methamphetamine and methaqualone detection in SUDI cases and it is unknown whether this lack is due to a lack of testing performed in the

field or due to methaqualone and methamphetamine administration (intentional or unintentional) or exposure to infants not being common. There is also limited knowledge concerning the disposition of these drugs in infants, which too could alter the detection in different biological samples.

Methaqualone is a member of the quinazolinone drug class and it is a central nervous system (CNS) depressant (Rossiter, 2017). Methaqualone was initially introduced as a therapeutic agent to treat insomnia and was described as non-addictive (Angelos & Meyers, 1985). In 1971, methaqualone was listed as a drug of abuse for the first time and thereafter its use was banned in many countries (Van Zyl, 2001). The South African Community Epidemiology Network on Drug Use (SACENDU) reported that in 2016, individuals who reported to specific treatment locations in the Western Cape did so most commonly for methamphetamine, followed by cannabis, alcohol and then methaqualone (South African Medical Research Council, 2017). The detection of methamphetamine and methaqualone in SUDI cases admitted to a Cape Town mortuary, was thus in alignment with the reported abuse of these drugs in the Western Cape Province of South Africa.

Maternal methaqualone use was not reported in any of the cases where methaqualone was detected (n = 9), which in turn raises the question: how was the infant exposed to the drug? Did exposure take place *in utero,* did exposure take place in its immediate environment after birth or did the mother or anyone else in the house use it (inhalation of second-hand smoke)? The mere presence of any drug of abuse in an infant should never be overlooked, and questions about possible routes of exposure should be considered. However, in two of the cases where methamphetamine was detected, did the mothers report use of the drug. The detection of methaqualone in hair samples together with the lack of self-reported maternal methaqualone use establishes the possibility that the drug was not intentionally administered to the infant but that environmental drug exposure could have led to incorporation of the drug in the infant's hair and subsequent detection (Papaseit et al., 2011).

Methamphetamine is a CNS stimulant (World Health Organisation, 2011; Lakhan & Kirchgessner, 2012; Chen et al., 2016). Methamphetamine exerts its toxicity mainly through increasing extracellular dopamine, serotonin and norepinephrine above normal levels (Shoar, Marwaha & Molla, 2019). It has shown to cause direct neurotoxicity to dopaminergic and serotonin neurons, as well as reduce grey matter (Krasnova & Cadet, 2009; Yu et al., 2015). Unlike cocaine and amphetamine, methamphetamine is directly toxic to midbrain dopamine

neurons (Malenka, Nestler & Hyman, 2009). It is a highly addictive drug that produces an effect similar to that of adrenaline and can be administered in a variety of ways including smoking (inhalation), orally (pill form), snorting or injection of dissolved powder (Anglin et al., 2000; National Institute on Drug Abuse, 2019). In 2010, Peltzer et al. reported that methamphetamine abuse in South Africa was the highest in the Western Cape province (Peltzer et al., 2010), which was reported for 2016 as well (South African Medical Research Council, 2017). This prevalent nature of methamphetamine and methaqualone was also reported within a deceased cohort of violent deaths admitted to SRM (Auckloo & Davies, 2019). These reported results are similar to the findings of the current study, which was conducted on a different cohort at the same medico-legal mortuary, and further illustrates the wide-spread presence of drugs in the community.

Since the majority of drugs of abuse were only detected in the hair samples of the nine cases, it is possible that the drug was either incorporated into the hair as an external contaminant that was not adequately removed during the washing step in the sample preparation procedure, or that the infant was exposed to these drugs at some earlier point in time, but not necessarily acutely, or it could possibly have been a combination of both factors (Papaseit et al., 2011). Environmental exposure may include exposure to drugs in the household environment through smoke for example. Even if the exposure was not intentional per se, the fact that the infant lived in an environment where they were exposed to drugs of abuse, may point to possible neglect. In South Africa it is necessary to notify the Department of Social Development (DSD) of the situation to ensure that necessary steps are being taken to provide for the safety of remaining children in the household.

In response to child mortality in the Western Cape, a Child Death Review (CDR) panel was piloted in 2014 and was subsequently formally implemented in the Western Cape and in some regions of Kwa-Zulu Natal (Matthews & Martin, 2016). At the CDR, the deaths of all individuals under the age of 18 years are retrospectively evaluated each month by a multidisciplinary panel consisting of agents from law enforcement, forensic pathology, DSD, the National Prosecution Authority (NPA) and the Department of Health (Matthews & Martin, 2016). Information specific to each of the cases under review as well as circumstances surrounding death are discussed and social workers present act upon any cases of possible child neglect and abuse (Matthews & Martin, 2016).

Following on from this study, if SUDI cases routinely undergo toxicological testing, the generated results may be brought to the CDR forum for discussion which should assist not only in cause/contribution to death determination, but also to provide evidence of possible neglect that may require further investigation. It is clear that without toxicology, this information may go unnoticed, particularly as self-reported drug use does not always correlate with the toxicological evidence.

Detecting drugs of abuse in nine cases in a study cohort of 30 individuals illustrates the importance of toxicological investigations in all SUDI cases admitted to SRM. It is important that these investigations become routine, even in cases where the cause of death seems to be natural, since the presence of drugs of abuse and their potential contribution to death, may be missed in the absence of toxicological testing. The presence of methaqualone in hair samples only also highlights the importance of incorporating routine hair analysis in the investigation of SUDI cases.

4.2 The value of hair as a sample type

Drugs were detected in all hair samples that underwent toxicological analysis, even in cases where no drugs were detected in the other sample types (Table 3.1). Further, some drugs (e.g. methaqualone) were only detected in the hair samples (Table 3.1). Overall, there were significantly more drugs detected in hair samples than in bodily fluids (Figure 3.3). This was expected as hair analysis, unlike bodily fluids, usually provides a longer history of exposure to drugs (weeks to years) and not just for the period immediately prior to death (Lappas & Lappas, 2016; Kintz, 2019).

The hair of a foetus starts to grow during the last trimester of pregnancy (approximately 20 weeks gestation), and an infant is born with that hair (Koren, 1995). Toxicological hair analysis in infants may therefore be useful to detect foetal (gestational) exposure to drugs since the infant may retain drugs that the he/she was exposed to during that period (Akiyama, Matsuo & Shimizu, 2000; Bar-Oz et al., 2003).

In the post-mortem setting, hair is sometimes the only specimen available in SUDI cases as other specimens (such as blood, urine, vitreous humour) may be limited and/or used for other ancillary investigations. Hair is also a much easier and non-invasive sample to collect compared to bodily fluids (Kintz, Villain & Cirimele, 2006). In this study, hair could still be

obtained in cases where the authorised medical practitioner did an external examination and not a full autopsy. Another advantage of hair as specimen of choice compared to other specimens is its resistance to degradation and decomposition. It can be stored for much longer before analysis and does not need special storage conditions, such as refrigeration (Kintz, 2019).

One key issue of post-mortem drug detection in hair is the high risk for false positive results as a result of passive drug exposure (Kintz, 2019). Passive drug exposure refers to the unintentional exposure to drugs through drug exposure in the environment, such as contact with a contaminated surface or hair contamination by smoke or other contaminants in the immediate environment (Kintz, 2019). This factor must be taken into account and positive drug detection in hair should be interpreted with caution and within the case context.

Researchers have therefore recommended at least one washing step as part of the sample preparation method in order to minimise the risk for false positive hair analysis results. The aim of the washing step is to remove any possible contaminants (mostly from passive environmental exposure) from the external surface of the hair samples (Kintz, 2019). The sample preparation method used in this study involved two washing steps in an attempt to remove contaminants (section 2.4.2.3). Drugs were not detected in the washings of the hair samples in this study and were only detected in the actual hair analysis (the extracted samples). This suggests that the drugs detected were incorporated into the hair, and therefore likely absorbed and distributed within the infant.

Information concerning self-reported maternal drug use may assist in interpreting post-mortem toxicological findings. The issue with this however, is that reported drug use is often not accurate, especially when it relates to abuse of drugs (Kintz, Villain & Cirimele, 2006). Therefore, maternal hair analysis has also been recommended, where the maternal hair is also analysed and results are compared to the hair analysis results from the infant. If there is a correlation with the self-reported drug use by the mother (both during pregnancy and post-partum), stronger conclusions may be drawn regarding the route of drug exposure (Allibe et al., 2015).

In this study, for two of the cases where methamphetamine was detected, self-reported maternal methamphetamine use was recorded, and it assisted with interpretation of toxicological results. In the remaining case where methamphetamine was detected and in none of the nine cases where methaqualone was detected, was any information on maternal drug use recorded. In

these cases, the information provided by the mother regarding drug use was inaccurate or the infant could possibly have been exposed to these drugs in its immediate environment. Since the most common method of administration of both methamphetamine and methaqualone is smoking (United Nations Office on Drugs and Crime (UNODC), 2019), it is possible that the infants were exposed to smoke in their immediate environments. If that is the case, response by a social worker should still be sought, as the presence of a drug of abuse may indicate an unsafe environment, where the risk for accidental exposure is high, particularly for other children still living in the household.

The results from this study highlighted the value of collecting and analysing hair samples together with other samples, not only to confirm drugs detected in the other samples, but to also provide valuable insights with regards to the infant's history of drug exposure; from the third trimester of pregnancy until death. This study highlights the opportunities for investigating the use of maternal hair analysis together with that of the infant. It also highlights the need for further work in investigating not only detection, but also quantitative concentrations of drugs in infant hair, and whether these may point towards different routes of exposure (e.g. passive inhalation from the environment versus ingestion through breast milk). Additionally, more value may have been be added if maternal drug use (therapeutic drugs and drugs of abuse) was accurately declared. However, it has been shown that maternal self-reporting is unreliable (Koren, 1995; Garcia-Bournissen et al., 2007), which correlates with what has been found in this study.

4.3 Self-reported maternal behaviour

4.3.1 Drug administration

There was a discordance between the drugs reportedly administered to the infant prior to death (by next-of-kin), and the drugs that were detected (Figure 3.7). Kintz et al. suggested that self-reported maternal drug use and/or reported drug administration to infants by next-of-kin are not reliable (Kintz, Villain & Cirimele, 2006). Of the six reported drugs that were allegedly administered to infants prior to death; only three, acetaminophen and antibiotics (sulfamethoxazole and ciprofloxacin), were detected during toxicological analysis. Although the presence of antibiotics was expected, it was not detected in any of the cases where the next-

of-kin reported antibiotic administration prior to death. Methamphetamine was reportedly abused by mothers in two cases but was detected in a total of three cases.

None of the other drugs (ARVs, PPIs, CCMs and multi-vitamins) administered to the infants, as indicated by the next-of-kin (Appendix C.1), were detected during the toxicological analysis. ARVs and vitamins are not screened for during the toxicological analysis and therefore it was not expected to be detected. However, guaifenesin, an expectorant which is used to treat cough symptoms, was detected in one case (4 %), but not reported as administered by the next-of-kin.

Based on the information provided by the next-of-kin of the study participants, the following drugs were not expected during toxicological analysis: all ten detected drugs not recommended for infant use (thiabendazole, quinine, hydrocortisone, guaifenesin, chlorpheniramine, ondansetron, lidocaine, tramadol, verapamil and caffeine), one of the drugs permissible for infant use (metoclopramide) and one of the drugs of abuse (methaqualone) (Figure 3.5).

Comparing the detected drugs with the drugs reported to have been administered to infants highlighted two issues: firstly, the unreliability of information provided by the next-of-kin regarding drugs administered to infants prior to death. It is however possible that in some of the cases where drugs were detected but not reported, this was due to the *accidental* exposure to drugs and therefore the next-of-kin may not have known. The second issue that was raised was the lack of information on therapeutic drugs used by the mother. Since it was possible that the infant could have been exposed to drugs *in utero* and that those drugs could possibly have been incorporated into the infant's hair it was important to know which drugs the mother used during that time. Since a wide range of drugs are secreted into breast milk, it is important to know which drugs the mother used while the infant was breastfed.

Garg et al. (2016) illustrated the limited validity of self-reported maternal drug abuse and reported that drug use was substantially underreported and not always reliable (Garg et al., 2016). The authors recommended that researchers should not rely solely on this information alone (Garg et al., 2016). The unreliability and/or underreporting of maternal use of drugs of abuse was illustrated in this study by the high level of drugs of abuse detected in infants (30 %) compared to the low level of self-reported maternal drug use (7 %).

4.3.2 Breastfeeding

Based on information provided by the next-of-kin, 40 % of the infants in the study population were breastfed (Appendix C.1). This information is important when interpreting post-mortem toxicological results. Knowing whether or not an infant was breastfed may assist in interpreting infant toxicology results by considering the possibility that detected drugs might have been transferred from the mother to the infant through breast milk. This information should thus be accurately recorded. In this study, numerous drugs, which can be secreted into breast milk, were detected in infants where the mother did not indicate if breastfeeding occurred. This included acetaminophen (10 cases), caffeine (eight cases), sulfamethoxazole (six cases), verapamil (nine cases), among others. The interpretation of these findings was therefore complicated, since it was not clear if infants were given these drugs, or if mothers were taking these drugs, and/or if the mother was breastfeeding.

Some drugs with the potential to be secreted into breast milk may be transferred via breast milk to the nursing infant and possibly produce adverse effects (Anderson, Pochop & Manoguerra, 2003). However, not all drugs that are used by breastfeeding mothers will be necessarily present in breast milk and/or in equal amounts. Therefore, the exposure of the infant to a specific drug will vary depending on its proportion in breast milk, which is affected a number of factors, including frequency and dose of maternal use, extent and rate of drug secretion into breast milk as well as the timing of breastfeeding (Sachs, 2013). The adverse effects that may occur as a result of drug transfer through breast milk also depends on the age of an infant, where it was reported that infants under the age of two months experienced more adverse effects than those older than six months which is likely as a result of pharmacokinetic differences between infants and older children (Anderson, Pochop & Manoguerra, 2003). This complexity of interpretation of post-mortem toxicology results thus highlights the importance of the information that needs to be provided by next-of-kin at the post-mortem investigation interview.

While the FPS006(b) form has been established and implemented to record relevant data from families of SUDI cases, it is perhaps not used optimally, as highlighted by previous research (Bennett, Martin & Heathfield, in press). In here, the researchers reported many instances for which multiple questions are left unanswered or some sections are left completely blank in the form and therefore made suggestions that the individuals who fill out the form needs to undergo appropriate training and/or the format of the current document needs to be revised (Bennett,

Martin & Heathfield, in press). In this study, an additional gap was identified in the FPS006(b) form, where there were insufficient questions pertaining to maternal drug use, especially as it relates to drugs other than those recreationally used or abused. A holistic picture of all drug use, including therapeutic medication use, would be ideal for supporting the interpretation of post-mortem toxicological results in SUDI cases.

4.4 The role of drugs and infection

A discordance between illness of the infant prior to death (as reported by the next-of-kin) and the cause of death as determined by the medical practitioner was noted in this study. Illness was reported in 30 % of study participants, while the medico-legal death investigation determined that 71 % of the SUDI cohort had infectious causes of death. Various drugs were found in infants with infectious causes of death. There was no significant association between the presence of antibiotics and infection as cause of death.

The results could suggest that the next-of-kin may not have recognised symptoms of illness in their infant and/or not have given appropriate medication. However, if the next-of-kin deliberately did not give medication, then this could also be viewed as negligence and further investigation should ensue (Matthews & Martin, 2016). Conversely, antibiotics were also detected in infants where administration was not reported by the parents, as well as in infants who were not ill. This detection may have been from maternal antibiotic use, which may have been transferred to the infant either through breast milk or exposure *in utero*, or from intentional administration by caregivers or from oversight on the part of the next-of-kin.

The burden of infectious causes of death in infants has previously been reported for Salt River Mortuary (Heathfield, Martin & Ramesar, in press; Matthews & Martin, 2016) and the results of this study also confirmed it. The burden of disease in the Western Cape, South Africa, was estimated to be almost three times higher than in developed countries (Househam, 2010). Drug and alcohol use has also been associated with a high burden of disease (Househam, 2010; GBD 2016 Alcohol and Drug Use Collaborators, 2018) for example the increased risk for infectious disease as a result of methamphetamine use (Salamanca et al., 2015).

For example, a risk factor and contributor to respiratory disease is exposure to smoke, either from cigarettes, air pollution or smoking of drugs of abuse (Zar & Ferkol, 2014). Further, in lower-income households, the quality of circulating air may be low, more people may live

together in a small area and some may smoke, which may put the children in the household at risk for developing respiratory infections (Torres-Duque et al., 2008; Zar & Ferkol, 2014).

This study highlights the importance of raising awareness about the importance of a safe household environment in limiting the risk of infants developing infection, particularly in the presence of drugs. Raising awareness regarding the dangers of smoking (nicotine and drugs of abuse) in the presence of infants and the importance of improving air circulation and air quality in the household environment is important (Zar & Ferkol, 2014). As is increasing awareness surrounding the transfer of drugs (medicinal and recreational) through breast milk.

4.5 Limitations

This study had several limitations, the first of which was the convenience sampling of the cohort. While it was deemed appropriate for the pilot nature of this study, it may have excluded other pertinent cases and may not have be representative of the SUDI population (at SRM) as a whole. Another 'confounding factor' in reporting true percentages of drugs detected, was that next-of-kin had the *option* to participate in the study. In some cases, the parents were approached for informed consent, but they declined to participate in the research study. Therefore, no samples were collected from these infants, and it remains unknown if there were drugs present in these infants. However, based on this preliminary data on this relatively small cohort, further research consisting of a larger sample size is strongly motivated.

Another limitation of this study was limited sample availability. In infants, bodily fluids were limited as a result of their smaller body size, and particularly in infants who were sick or dehydrated prior to death. Post-mortem changes also make obtaining blood samples challenging, and the availability of these and urine was beyond the researchers' control. Insufficient amounts of sample collected also resulted in only one analysis being possible for some samples. If this failed, then no further testing could be done. This resulted in incomplete results and therefore a lack of information in four cases. This also poses a challenge in future testing where more comprehensive analyses (on different instruments for example) may be required.

A limitation with regards to sample analysis was the inability of the instrument to screen for the presence of ARVs and multivitamins. The instrument did not screen for these drugs since they were not included in the library. This resulted in these drugs not being detected even if

they were present in the cases where the administration of these drugs was reported. Their absence thus does not mean that these drugs were not present. This limitation also applies to other drugs: the method used in this study expands to a large number of substances but is still reliant on library identification which may not include certain drugs. Sample preparation may not be optimal to extract certain drugs. This could be improved in future by including a GCMS screen, especially for some basic drugs, and optimising the extraction techniques used.

Lastly, the toxicological analysis provided qualitative and not quantitative results. While the qualitative screen was sufficient in indicating whether a drug was present or not, the mere presence of a drug does not provide enough information to draw direct conclusions about the role of the drug in death. Therefore, future research should consider both qualitative and quantitative toxicological analyses of the samples.

4.6 Recommendations for future research

At autopsy, adequate amounts of available biological samples should be collected wherever possible. This is important to ensure that the amount of sample is sufficient for analysis and possible re-analysis. This is especially important in cases where hair is the only available sample. To ensure that adequate amounts of sample are collected, it may be useful to establish guidelines for minimum amounts required for analysis. This may then be used to guide the medical practitioners to ensure collection of adequate amounts of sample.

It would be ideal to follow the qualitative toxicological drug screen of samples collected from SUDI cases by quantitative confirmatory testing, at least in blood. During the quantitative analysis concentrations of the detected drugs are generated. The concentrations of detected drugs may provide more insight into the levels of exposure. When concentrations of drugs are produced, it may be compared to peer-reviewed published data. This may aid in a more reliable interpretation of the detected drugs and more conclusions can be drawn compared to only being able to report the presence of drugs. This will still be challenging though as there is limited published data concerning drug concentrations in infants. These analyses could therefore contribute to a growing literature base.

It is recommended that additional information pertaining to maternal use of therapeutic drugs, both during pregnancy and thereafter, is documented. Recording the dose as well as whether the drugs were acutely used or chronically used, may provide more insight when interpreting

toxicology results. It is also recommended that historic maternal use of drugs of abuse are documented to interpret the presence of drugs of abuse more accurately in infant hair. It is suggested that this is also amended in the FPS006(b) form for routine service delivery.

Lastly, maternal hair testing may provide an interesting and useful avenue of research in SUDI cases. This may prove to be useful in cases where the mother used either therapeutic drugs or drugs of abuse. The results of maternal hair testing may be used to establish an association between what was seen in infant hair and the history as reported by the mother (Nakhara, 1999). In these cases it is also important to collect sufficient amounts of sample for multiple analyses (Nakhara, 1999).

4.7 Conclusion

This study was the first at SRM to prospectively collect samples to investigate the presence of drugs in SUDI cases admitted for medico-legal investigations. The results of this study supported the hypothesis that drugs would be detected in the SUDI cases, which ordinarily would not have undergone any toxicological testing. Drugs of abuse were detected in 30 % of infants, which highlighted the valuable role of toxicological testing in SUDI cases as this information would otherwise go unnoticed. This study further emphasised the importance of hair samples for toxicological investigation, especially in investigating long-term drug exposure. These findings illustrate the value of toxicological testing and provide support for the routine incorporation of toxicological analysis in the vulnerable SUDI population, which may assist in determining cause of death but also identifying siblings who may be at risk for neglect in certain households. Future work in this field should include quantitative drug analysis in a larger cohort of SUDI cases and an investigation into the role of toxicological outcomes to assist social development interventions.

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Appendices

Appendix A.1: UCT HREC Ethics approval letter

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

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

12 October 2015

HREC REF: 445/2015

Prof R Ramesar
Human Genetics
FHS

Dear Prof Ramesar

PROJECT TITLE: SUDDEN UNEXPECTED DEATH IN INFANTS: POTENTIAL GENETIC CONTRIBUTIONS IN A SOUTH AFRICAN COHORT-(PhD-candidate-L Heathfield)

Thank you for your response letter addressing the issues raised by the Human Research Ethics Committee (HREC).

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

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

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

We acknowledge that the following student: - Laura Heathfield is also involved in this project.

Please quote the HREC reference no in all your correspondence.

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

Yours sincerely

Signature removed to avoid exposure online

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research

Appendix A.1: UCT HREC Ethics approval letter (continued)

Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The 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:445/2015

Appendix A.1: UCT HREC Ethics approval letter (continued)

HUMAN RESEARCH ETHICS COMMITTEE
17 APR 2019
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Form FHS007: Amendment – study staff

HREC office use only (FWA00001637; IRB00001938)			
☐ Approved			
This serves as notification that all changes to the study staff and documentation described below are approved.			
Chairperson of the HREC signature	signature removed	Date	17/4/2019

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	04/03/2019		
HREC REF Number	445/2015		
Protocol title	Sudden unexpected death in infants: a forensic genetics investigation in a South African cohort		
Protocol number (if applicable)			
Principal Investigator	Raj Ramesar		
Department / Office Internal Mail Address	Reception, Division of Forensic Medicine and Toxicology, Falmouth Building (entrance 3, level 1), Faculty of Health Science, UCT, Anzio Road, Observatory		
1.1 Does this protocol receive US Federal funding?		☐ Yes	☐✓ No

2.1 Staff changes (tick ✓)

Are new personnel being added to this research?	☐✓ Yes	☐ No
Are current personnel being removed from this research?	☐✓ Yes	☐ No
Is the principal investigator for this research being changed? If yes, please attach revised conflict of interest and PI declaration statements. (Refer: sections 7 and 8.3 in the New Protocol Application Form - FHS013)	☐ Yes	☐✓ No
Do the consent and assent forms need modification to reflect these staff changes? If yes, please attach copies of the revised forms, with all changes highlighted or tracked and listed in the documents for approval.	☐✓ Yes	☐ No

Appendix A.1: UCT HREC Ethics approval letter (continued)

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

2.2 Amended study staff details

Title, first name, surname	Department/Division	E-mail	Role of new staff member
Ané Louw	Pathology, Forensic Medicine and Toxicology	LWXANE002@myuct.ac.za	Co-investigator
Bronwen Davies	Pathology, Forensic Medicine and Toxicology	bronwen.davies@uct.ac.za	Co-investigator
Sairita Maistry	Pathology, Forensic Medicine and Toxicology	Sairita.Maistry@sswahs.nsw.gov.au	None - Immigrated

3. List of documentation for approval

Please list below all staff documentation such as CVs, declarations, GCP certificates and revised consent forms which need approval. This information must correspond to all 'yes' answers in 2.1 above. This form will be signed and returned to the PI as notification of approval. Please add extra pages if necessary.

CVs of two new co-investigators are attached.
Sairita Maistry has immigrated to Australia and is no longer involved in this project.
Revised consent forms attached.

4. Signature

My signature certifies that I will maintain the anonymity and/ or confidentiality of information collected in this research. If at any time I want to share or re-use the information for purposes other than those disclosed in the original approval, I will seek further approval from the HREC.

Signature of PI	Signature removed	Date	04/03/2019

Appendix B.1: Informed consent form

INFORMATION FORM AND INFORMED CONSENT FORM

INFORMATION FORM

Study title: Sudden unexpected death in infants: a forensic genetic investigation in a South African cohort

Principal investigator: Laura Heathfield
Co-investigators: Prof Raj Ramesar, Prof Lorna Martin, Bronwen Davies and Ané Louw

Introduction to the study
You are invited to participate in research study which is a collaborative project between the Divisions of Human Genetics and Forensic Medicine at the University of Cape Town.

It will be conducted by Laura Heathfield and Ané Louw who are researchers in the Division of Forensic Medicine at the University of Cape Town.

The purpose of the research study is to try and understand why some infants experience sudden and unexpected death. 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 make a decision about participating.

Background
Sudden unexpected death of infants is a devastating loss and is often caused by infections, inborn errors of metabolism or environmental factors (such as exposure to drugs) that surround an infant at a vulnerable time of their natural development. Sometimes after post-mortem investigation, the cause of death still cannot be determined. This suggests that maybe there are genetic variations that may contribute to the death of the infant or predispose them to contracting an infection. It is also possible that they could have been exposed to drugs or medication unintentionally or without the knowledge of their parents or primary caregiver.

All humans are made up of many cells. Each cell contains DNA which is made up of genes. It is these genes that contain information on how a person will look or whether they get a certain disease. Sometimes these genes may predispose someone to be more vulnerable to an environmental factor, such as getting an infection or not being able to sleep on their tummy. It might also affect the way they process drugs and medication. This study wants to investigate what genetic factors could be responsible for causing a sudden and unexpected death of an infant less than one year old.

In this investigation, it is also important to know if babies were exposed to any drugs or toxins which could have caused their death. This exposure may have been on purpose or completely by accident. Sometimes the doctor gives the baby medication (drugs) but the baby cannot process it properly. Testing for the presence of drugs or toxins is not commonly performed on

babies in South Africa. However, if drugs are found, then it helps the scientists know what genetic tests should be done. The information can be used to highlight the importance of doing these tests in all of these cases.

To be able to do these investigations, different biological samples such as blood, urine, eye fluid and hair must be collected from infants who experience a sudden and unexpected death. The genetic material will be extracted and analysed which will help to find genetic variations that may have contributed to the cause of the death. The samples will also have an analysis where the presence of drugs and medication can be detected. Once this research has been done and validated, this information will eventually be able to help determine the cause of death of other infants who experience sudden unexpected death and may even help family members in case they want more children.

Procedure

You will be asked to give permission to allow various samples to be collected at the autopsy by the Forensic Pathologist from your deceased family member:

- 2 x 5 ml samples of blood
- 1 x urine sample
- 1 x sample of eye fluid (vitreous humour)
- 1 x hair sample

Once samples are collected, there will be laboratory tests on the samples. DNA will be extracted from one of the blood samples and studied more closely (for e.g. DNA sequencing and genotyping). All of the other samples will be tested for the drugs and medications. Please note that technology in the laboratories are always getting better so in time there might be newer laboratory techniques invented that may become accessible to the researchers. You will also be asked to allow the biological samples collected to be used in these newer technologically advanced methods if and when they become available.

The biological material collected from your deceased family member and the extracted DNA will be stored for a period of 20 years at the University of Cape Town. After this time the samples will be appropriately discarded.

The name of your family member will not be made known. Instead the samples, extracted DNA and generated data will be coded 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 will be required to fill in a confidentiality agreement.
2. Only the primary investigators will have access to the database.
3. All samples, genetic data and results generated will be stored in locked filing cabinets and password protected program at the University of Cape Town.
4. Sharing of experimental data will be done on the basis of alphabetical and numerical codes.

5. 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.
6. Your participation will be kept confidential.

The biological samples will not be used for any research unless the research study is reviewed and approved by the University of Cape Town, Faculty of Health Sciences, Human Research Ethics Committee. This committee is responsible for protecting the rights and welfare of individuals who volunteer for participation in research studies.

There are several things you need to know before allowing biological samples to be taken from your deceased family member:

1. When research is carried out it is not the policy of the University of Cape Town to provide genetic information about the deceased to the family members.

2. Participation in this study is voluntary. You are free not to participate or to withdraw at any time, for whatever reason, without negative consequences. In such cases no samples will be collected and your choice will not affect the way you will be treated at the Salt River Mortuary.

3. If you participate in the study, you can change your mind later and decide that you don't want to participate anymore and you do not want the tissue to be used in this study. Please let us know and we will destroy the samples. If the sample has already been analysed at the time you change your mind, your results and other data may have already been shared with other investigators. In that case, we will not be able to destroy this data. The data will be removed from the secured database. That means that no additional researchers can get the data.

4. You will not receive feedback of possible genetic variations that are found in the DNA of the deceased or whether drugs or toxins have been detected in the samples. This study is unlikely to benefit you or your family directly, but it is hoped that it will contribute to knowledge about sudden unexpected death in infants in the future.

5. There will be no cost to you and there will no compensation for your participation.

Making your choice

Please read each sentence below and think about your choice. 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 may have any questions with regards to the rights and welfare of a research subject in the study, please contact the

Chairperson of the University of Cape Town Faculty of Health Science Human Research Ethics Committee, **Professor Marc Blockman** on (021) 406 6496. If you require any further information about this study please contact Laura Heathfield at (021) 406 6569 or email at laura.heathfield@uct.ac.za

If the spouse/partner/major child/parent/guardian/major sibling agrees then the consent form needs to be read and informed consent will be taken. **Please note that the information and consent forms will be translated into the family member's language of choice.**

- Thank you for your time –

Please find attached the consent form to be signed if you wish to proceed.

CONSENT FORM

I, _____ (full name), the spouse/partner/major child/parent/guardian/major brother/major sister (circle relationship) of the deceased; fill in the Western Cape death register number: WC11

I confirm that I have:	**Yes**	**No**
a) Read and understood contents of this form and agree to be a part of the research study.		
b) Been informed about this study's purpose, procedures, possible benefits, and risks.		

I give consent and agree that:	**Yes**	**No**
a) Biological samples in the form of blood can be taken from my deceased family member for the genetic study.		
b) Biological samples in the form of blood, urine, eye fluid and hair can be taken from my deceased family member for the drugs and medication tests.		
c) The biological samples can be subjected to laboratory tests at the University of Cape Town in which samples will be analysed to better understand sudden unexpected death of infants.		
d) Agree that the samples and extracted DNA may be stored at the University of Cape Town for a period of 20 years after which it will be appropriately discarded.		
e) The stored genetic material and biological samples may only be used further research studies which have been reviewed and approved by the University of Cape Town, Faculty of Health Sciences, Human Research Ethics Committee.		
f) I may be contacted in the future by someone from the University of Cape Town who may ask me to take part in research that may develop from the results of the study.		

I further understand that:	**Yes**	**No**
a) 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.		
b) The scientific laboratories are **under obligation** to respect medical confidentiality.		
c) This study is unlikely to benefit me or my family directly.		
d) I can at any time withdraw my consent and that I have to notify the primary investigator of my decision to withdraw.		

e) Research conducted with this DNA and biological samples may result in publication, but neither the deceased nor the family of the deceased will be identified.		

I, have explained to _____(full name) who is the spouse/partner/major child/parent/guardian/major brother/major sister of the deceased; the purpose, procedures, possible benefits and discomfort of this research study; and how the samples will be collected and stored in the Division of Human Genetics at the University of Cape Town for use in the study and in possible further research by other individuals within the Division of Human Genetics at the University of Cape Town.

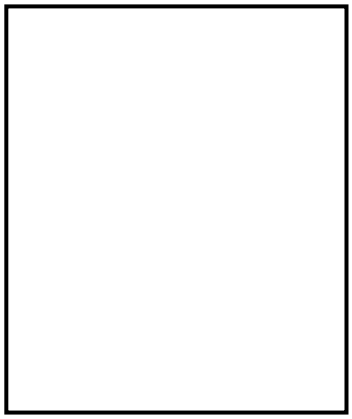
Full name of person obtaining consenting

Signature of person obtaining consenting Date

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

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

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



Full name of witness

Signature of witness Date

Appendix B.2: Delayed informed consent form

INFORMATION FORM AND DELAYED INFORMED CONSENT FORM

INFORMATION FORM

Study title: Sudden unexpected death in infants: a forensic genetic investigation in a South African cohort

Principal investigator: Laura Heathfield
Co-investigators: Prof Raj Ramesar, Prof Lorna Martin, Bronwen Davies and Ané Louw

Introduction to the study

You are invited to participate in research study which is a collaborative project between the Divisions of Human Genetics and Forensic Medicine at the University of Cape Town.

It will be conducted by Laura Heathfield and Ané Louw who are researchers in the Division of Forensic Medicine at the University of Cape Town.

The purpose of the research study is to try and understand why some infants experience sudden and unexpected death. 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 make a decision about participating.

Background

Sudden unexpected death of infants is a devastating loss and is often caused by infections, inborn errors of metabolism or environmental factors (such as exposure to drugs) that surround an infant at a vulnerable time of their natural development. Sometimes after post-mortem investigation, the cause of death still cannot be determined. This suggests that maybe there are genetic variations that may contribute to the death of the infant or predispose them to contracting an infection. It is also possible that they could have been exposed to drugs or medication unintentionally or without the knowledge of their parents or primary caregiver.

All humans are made up of many cells. Each cell contains DNA which is made up of genes. It is these genes that contain information on how a person will look or whether they get a certain disease. Sometimes these genes may predispose someone to be more vulnerable to an environmental factor, such as getting an infection or not being able to sleep on their tummy. It might also affect the way they process drugs and medications. This study wants to investigate what genetic factors could be responsible for causing a sudden and unexpected death of an infant less than one year old.

In this investigation, it is also important to know if babies were exposed to any drugs or toxins which could have caused their death. This exposure may have been on purpose or completely by accident. Sometimes the doctor gives the baby medication (drugs) but the baby cannot process it properly. Testing for the presence of drugs or toxins is not commonly performed on

babies in South Africa. However, if drugs are found, then it helps the scientists know what genetic tests should be done. The information can be used to highlight the importance of doing these tests in all of these cases.

To be able to do these investigations, different biological samples such as blood, urine, eye fluid and hair must be collected from infants who experience a sudden and unexpected death. The genetic material will be extracted and analysed which will help to find genetic variations that may have contributed to the cause of the death. The samples will also have an analysis where the presence of drugs and medication can be detected. Once this research has been done and validated, this information will eventually be able to help determine the cause of death of other infants who experience sudden unexpected death and may even help family members in case they want more children.

Procedure

You will be asked to give permission to allow various samples to be collected at the autopsy by the Forensic Pathologist from your deceased family member:

- 2 x 5 ml samples of blood
- 1 x urine sample
- 1 x sample of eye fluid (vitreous humour)
- 1 x hair sample

Once samples are collected, there will be laboratory tests on the samples. DNA will be extracted from one of the blood samples and studied more closely (for e.g. DNA sequencing and genotyping). All of the other samples will be tested for the drugs and medications. Please note that technology in the laboratories are always getting better so in time there might be newer laboratory techniques invented that may become accessible to the researchers. You will also be asked to allow the biological samples collected to be used in these newer technologically advanced methods if and when they become available.

The biological material collected from your deceased family member and the extracted DNA will be stored for a period of 20 years at the University of Cape Town. After this time the samples will be appropriately discarded.

The name of your family member will not be made known. Instead the samples, extracted DNA and generated data will be coded 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:

7. Every person involved in the project will be required to fill in a confidentiality agreement.
8. Only the primary investigators will have access to the database.
9. All samples, genetic data and results generated will be stored in locked filing cabinets and password protected program at the University of Cape Town.
10. Sharing of experimental data will be done on the basis of alphabetical and numerical codes.

11. 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.
12. Your participation will be kept confidential.

The biological samples will not be used for any research unless the research study is reviewed and approved by the University of Cape Town, Faculty of Health Sciences, Human Research Ethics Committee. This committee is responsible for protecting the rights and welfare of individuals who volunteer for participation in research studies.

There are several things you need to know before allowing biological samples to be taken from your deceased family member:

6. Biological samples are sometimes collected as a matter of routine during autopsy and are stored in the fridge, should they be required for any routine analysis. Sometimes the identification of a family member is carried out *after* autopsy and therefore these samples have already been collected.

7. These samples have not yet been touched for research purposes as we require permission from you to use these samples in our project. If you don't give us permission, then the samples will not be used for research purposes and will be destroyed accordingly. This will in no way affect the results of the autopsy and will have no negative consequence whatsoever. If you do give us permission to use these samples, then this will in no way affect the routine analysis that may need to be carried out on the samples already collected. It is completely voluntary to participate in this project.

8. When research is carried out it is not the policy of the University of Cape Town to provide genetic information about the deceased to the family members.

9. Participation in this study is voluntary. You are free not to participate or to withdraw at any time, for whatever reason, without negative consequences. In such cases no samples will be collected and your choice will not affect the way you will be treated at the Salt River Mortuary.

10. If you participate in the study, you can change your mind later and decide that you don't want to participate anymore and you do not want the tissue to be used in this study. Please let us know and we will destroy the samples. If the sample has already been analysed at the time you change your mind, your results and other data may have already been shared with other investigators. In that case, we will not be able to destroy this data. The data will be removed from the secured database. That means that no additional researchers can get the data.

11. You will not receive feedback of possible genetic variations that are found in the DNA of the deceased or whether drugs or toxins have been detected in the samples. This

study is unlikely to benefit you or your family directly, but it is hoped that it will contribute to knowledge about sudden unexpected death in infants in the future.

12. There will be no cost to you and there will no compensation for your participation.

Making your choice

Please read each sentence below and think about your choice. 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 may have any questions with regards to the rights and welfare of a research subject in the study, please contact the Chairperson of the University Of Cape Town Faculty Of Health Science Human Research Ethics Committee, **Professor Marc Blockman** on (021) 406 6496. If you require any further information about this study please contact Laura Heathfield at (021) 406 6569 or email at laura.heathfield@uct.ac.za

If the spouse/partner/major child/parent/guardian/major sibling agrees then the consent form needs to be read and informed consent will be taken. **Please note that the information and consent forms will be translated into the family member's language of choice.**

- Thank you for your time -

Please find attached the consent form to be signed if you wish to proceed.

CONSENT FORM

I, _____ (full name), the spouse/partner/major child/parent/guardian/major brother/major sister (circle relationship) of the deceased; fill in the Western Cape death register number: WC11

I confirm that I have:	**Yes**	**No**
c) Read and understood contents of this form and agree to be a part of the research study.		
d) Been informed about this study's purpose, procedures, possible benefits, and risks.		

I give consent and agree that:	**Yes**	**No**
g) Biological samples in the form of blood can be taken from my deceased family member for the genetics study.		
h) Biological samples in the form of blood, urine, eye fluid and hair can be taken from my deceased family member for the drugs and medication tests.		
i) The biological samples can be subjected to laboratory tests at the University of Cape Town in which samples will be analysed to better understand sudden unexpected death of infants.		
j) Agree that the samples and extracted DNA may be stored at the University of Cape Town for a period of 20 years after which it will be appropriately discarded.		
k) The stored genetic material and biological samples may only be used further research studies which have been reviewed and approved by the University of Cape Town, Faculty of Health Sciences, Human Research Ethics Committee.		
l) I may be contacted in the future by someone from the University of Cape Town who may ask me to take part in research that may develop from the results of the study.		

I further understand that:	**Yes**	**No**
f) 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.		
g) The scientific laboratories are **under obligation** to respect medical confidentiality.		
h) This study is unlikely to benefit me or my family directly.		
i) I can at any time withdraw my consent and that I have to notify the primary investigator of my decision to withdraw.		

j) Research conducted with this DNA and biological samples may result in publication, but neither the deceased nor the family of the deceased will be identified.		

I, have explained to _____(full name) who is the spouse/partner/major child/parent/guardian/major brother/major sister of the deceased; the purpose, procedures, possible benefits and discomfort of this research study; and how the samples will be collected and stored in the Division of Human Genetics at the University of Cape Town for use in the study and in possible further research by other individuals within the Division of Human Genetics at the University of Cape Town.

_____

Full name of person obtaining consenting

_____

Signature of person obtaining consenting Date

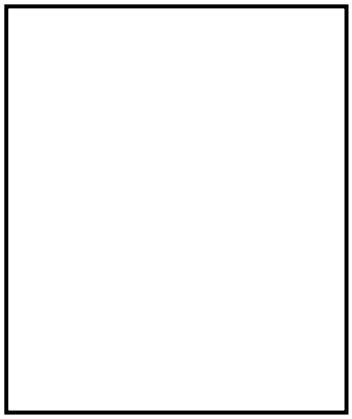
_____

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

_____

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

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



_____

Full name of witness

Signature of witness Date

Appendix C.1: Demographic information, social and clinical history and cause of death

Table C.1: *Demographic information and information regarding the burden of disease of the study population*

ID number	Age (months)	Sex	Ill prior to death	Received medical care	Medication administered	Maternal drug use	Manner of death	Cause of death	Type of death	Type of feeding
001	4	Female	Yes	No	None	None	Natural	Lower RTI	Infection (respiratory)	Bottle (formula)
002	5	Male	Yes	Clinic	Not recorded	None	Natural	Severe dehydration due to acute gastro-enteritis	Dehydration	Bottle (formula)
003	2	Male	No	No	Not recorded	Not recorded	Natural	Natural	UI	Bottle (formula)
004	0.75	Female	No	No	Not recorded	Not recorded	UI	UI	UI	Not recorded
005	1	Female	No	No	None	None	Natural	Sepsis	Infection	Breastfeeding
006	1	Female	No	No	None	Methamphetamine	Natural	Natural	Infection (respiratory)	Breastfeeding
007	4	Male	No	Clinic	None	None	Natural	RTI	Infection (respiratory)	Breastfeeding Bottle (formula)
008	2	Female	No	No	Nevirapine	None	Natural	Lower RTI	Infection (respiratory)	Breastfeeding
009	2	Male	Yes	Doctor	None	None	Natural	Likely meningitis	Infection (nervous)	Bottle (formula)
010	2	Female	No	No	Not recorded	None	UI	UI	UI	Breastfeeding
011	2	Female	Yes	Clinic	Panado; antibiotic	None	Natural	Lower RTI	Infection (respiratory)	Bottle (formula)
012	1	Male	No	No	None	None	Natural	RTI	Infection (respiratory)	Breastfeeding
013	2	Female	Not recorded	Not recorded	Not recorded	Not recorded	Natural	Dehydration	Dehydration	Breastfeeding
014	4	Female	Yes	Clinic	Panado; antibiotic	None	Natural	GIT infection	Infection (GIT)	Bottle (formula)
015	3	Female	No	No	Panado; multivitamin	None	Natural	Not recorded	Infection (respiratory)	Breastfeeding
016	7	Female	No	Not recorded	None	None	UI	UI	UI	Bottle (formula)
017	2	Male	No	No	None	Methamphetamine	UI	UI	UI	Bottle (formula)
018	6	Female	No	No	None	None	Natural	Lower RTI	Infection (respiratory)	Bottle (formula)

Table C.1 (Continued)

019	4	Male	No	No	None	Not recorded	Natural	RTI	Infection (respiratory)	Breastfeeding
020	3	Female	No	Clinic	None	Not recorded	Natural	Natural	Infection (sepsis)	Bottle (formula)
021	3	Female	No	No	None	Not recorded	Natural	Lower RTI	Infection (respiratory)	Mixed feeding
022	4	Male	Yes	Doctor; pharmacy	Panado; Amoxicillin; Calpol	None	Natural	RTI	Infection (respiratory)	Bottle (formula)
023	0.5	Male	No	No	None	None	Natural	Sepsis	Infection (sepsis)	Breastfeeding
024	3	Male	Yes	Pharmacy	Prospan	None	Natural	Pneumonia	Infection (respiratory)	Mixed feeding
025	5	Female	No	No	None	None	UI	UI	UI	Bottle (formula)
026	4	Male	Yes	Not recorded	Not recorded	None	Accident	UI	Asphyxia-aspiration	Mixed feeding
027	1	Male	No	Not recorded	None	Not recorded	Natural	Lower RTI	Infection	Breastfeeding
028	6	Male	Yes	No	None	None	UI	UI	UI	Mixed feeding
029	0.1	Male	No	No	ARVs	None	Natural	Congenital cardiac abnormality	Cardio-respiratory	Not recorded
030	2	Female	No	No	Panado; Caprol	None	Natural	Lower RTI	Infection (respiratory)	Breastfeeding Bottle (formula)

(ARVs: antiretrovirals; RTI: respiratory tract infection; UI: under investigation; mixed feeding: includes solid foods, not breast milk; Prospan: cough and cold medication)

Appendix C.2: High-resolution LC-QTOF targeted screening results

Table C.2: *Drugs detected during toxicological screening in blood, vitreous humour and urine*

ID number	Samples			
	Blood	**Vitreous Humour**	**Urine**	**Hair**
001	Sulfamethoxazole	Sulfamethoxazole	N/A	Sulfamethoxazole
002	None detected	N/A	None detected	Sulfamethoxazole
003	Acetaminophen Sulfamethoxazole	Acetaminophen Sulfamethoxazole	Acetaminophen Sulfamethoxazole	Acetaminophen Sulfamethoxazole Caffeine Verapamil
004	None detected	N/A	N/A	Acetaminophen Caffeine Verapamil
005	None detected	N/A	None detected	N/A
006	Methamphetamine	Methamphetamine	N/A	Caffeine Methaqualone
007	None detected	None detected	None detected	N/A
008	Sulfamethoxazole	Sulfamethoxazole	N/A	Sulfamethoxazole Caffeine Verapamil Methaqualone Tramadol
009	Acetaminophen	Acetaminophen Chlorpheniramine Metoclopramide	N/A	N/A
010	None detected	N/A	N/A	Caffeine Methaqualone Metoclopramide
011	N/A	N/A	N/A	IS error
012	N/A	N/A	N/A	IS error
013	N/A	N/A	N/A	Sulfamethoxazole Acetaminophen Caffeine
014	Acetaminophen	N/A	N/A	Acetaminophen Tramadol Thiabendazole
015	N/A	Acetaminophen Sulfamethoxazole Guaifenesin Hydrocortisone	N/A	N/A
016	None detected	None detected	N/A	Caffeine Verapamil
017	None detected	None detected	N/A	Methamphetamine Methaqualone Verapamil
018	None detected	N/A	N/A	Acetaminophen Caffeine Verapamil Lidocaine
019	Acetaminophen Sulfamethoxazole	Acetaminophen Sulfamethoxazole Ondansetron	Acetaminophen Sulfamethoxazole	Acetaminophen Sulfamethoxazole Caffeine Methaqualone Verapamil Quinine

Table C.2 (Continued)

020	N/A	None detected	N/A	Sulfamethoxazole Ondansetron Verapamil Tramadol
021	Sulfamethoxazole	Sulfamethoxazole	N/A	Sulfamethoxazole Acetaminophen Methamphetamine Methaqualone Caffeine Verapamil
022	Acetaminophen	Ciprofloxacin	N/A	N/A
023	None detected	N/A	None detected	IS error
024	Acetaminophen	Acetaminophen	N/A	N/A
025	None detected	N/A	N/A	Acetaminophen Caffeine Verapamil
026	Acetaminophen	Acetaminophen	N/A	IS error
027	None detected	N/A	N/A	Sulfamethoxazole Acetaminophen Methaqualone Caffeine Quinine
028	None detected	None detected	None detected	Acetaminophen Methaqualone Caffeine Verapamil
029	Caffeine	N/A	Caffeine	Sulfamethoxazole Acetaminophen Caffeine
030	Acetaminophen	Acetaminophen	Acetaminophen	Acetaminophen Methaqualone Caffeine Lidocaine Verapamil

(N/A: no sample available for analysis; internal standard (IS): RESTEK mixture of doxepin-d3 and diazepam-d5 detected in all samples; IS error: IS not detected during analysis)

Appendix C.3: Example of LC-QTOF targeted screening output

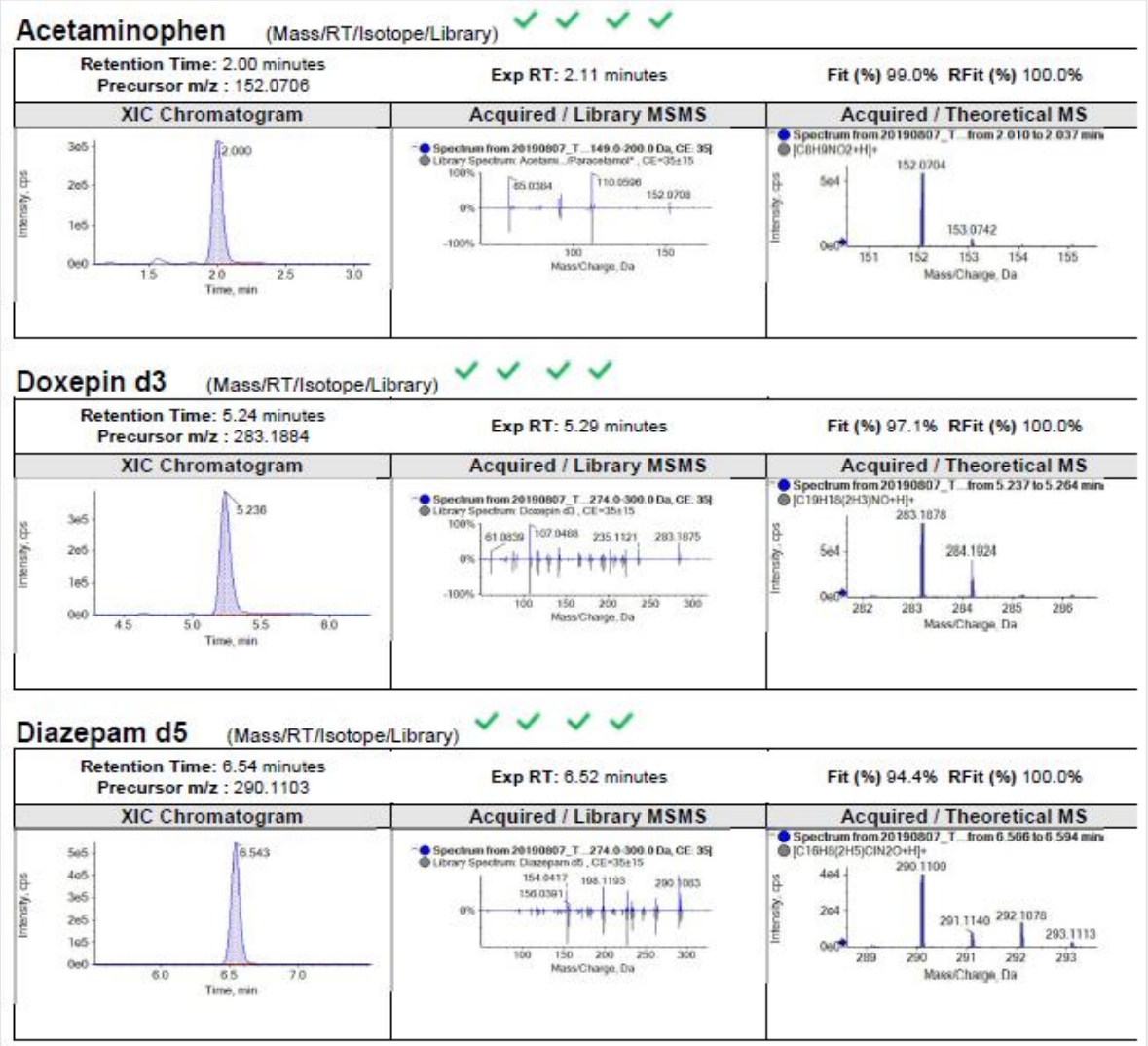


Figure C.1: *Toxicology screen results for the blood sample of case 009. Acetaminophen was the only drug detected. Doxepin-d3 and diazepam-d5 are the components of the IS. The green tick marks indicate that all four variables passed and the presence of the drug can be confirmed. (XIC chromatogram: illustrates retention time of drug (x-axis) and intensity (y-axis); Acquired/Library MS: illustrates key fragments of detected drug and compare it to the library spectrum; Acquired/Theoretical MS: shows the mass spectra of both the drug and the library).*