WASTEWATER-BASED DRUG EPIDEMIOLOGY TO ESTIMATE SOCIETAL DRUG USE
A CRITICAL REVIEW

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Declaration

I declare that this thesis does not contain any material submitted previously for the award of any other degree or diploma at any university or other tertiary institution. Furthermore, to the best of my knowledge, it does not contain any material previously published or written by another individual, except where due reference has been made in the text. Finally, I declare that all reported experimentations performed in this research were carried out by myself, except that any contribution by others, with whom I have worked is explicitly acknowledged.

Signed:

Lena Tran

20/07/2018
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PART ONE

Literature Review

Wastewater-based Drug Epidemiology to Estimate Societal Drug Use:

A Critical Review
1. ABSTRACT

Illicit drug use has many consequences resulting in social, health, and economic harm. An objective method of quantifying societal drug use would be useful for the efficient directing of the efforts of law enforcement, medical facilities and policy makers, and to inform the community. To this end, efforts to ascertain societal drug use have relied upon community surveys and extrapolation from law enforcement seizures, which often present the data as biased or skewed due to a small sample size and other associated limitations.

In recent times, wastewater-based drug epidemiology (WBDE) has been proposed as a suitable means to objectively quantify societal drug use. WBDE is the study of the incidence and distribution of drug use within a population and the its factors affecting the health and welfare. It is a method contingent upon the concept of measuring drug metabolites or biomarkers in wastewater (WW), from which levels of societal drug use are estimated and quantified through extrapolation and back-calculations.

The aim of this study was to critically review the various methods of WBDE that have been applied in Australia and in Europe. The outcomes of the assessment pertaining to their validity in directly and objectively measuring societal drug use will be presented.

Keywords: wastewater-based drug epidemiology, wastewater-based epidemiology, illicit drugs, drug abuse, estimate, societal drug use, population
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4. ABBREVIATIONS

DTR......................................................................................................................Drug target residues
LC-MS/MS..................................................................................................Liquid Chromatography coupled with tandem mass spectrometry
MS.....................................................................................................................Mass spectrometry
NDSHS...................................................................................................National Drug Strategy Household Survey
NPS..............................................................................................................Novel psychoactive substances
PLE.............................................................................................................Pressurised liquid extraction
SPE..............................................................................................................Solid phase extraction
SPM................................................................................................................Suspended particulate matter
WBE............................................................................................................Wastewater-based epidemiology
WBDE........................................................................................................Wastewater-based drug epidemiology
WW...............................................................................................................Wastewater
WWTP......................................................................................................Wastewater treatment plant
Illicit drug use has been long debated and plagued society, especially in the modern world with new drugs surfacing rapidly. While some argue the beneficial and medicinal effects of some drugs, particularly cannabis\textsuperscript{1,2}, typically, illicit drug use has a negative effect on society, and is viewed as being responsible for immense social, medical, policing, and economic burdens. Illicit drug use in the community has been shown to affect the individual’s interaction and connection to society, often resulting in detrimental disconnection from the world around them. Drug use not only affects the user, but also their family and friends, extending out to the community around them.

Illicit drug abuse has been shown to stabilise across the recent years, the National Drug Strategy Household Survey (NDSHS) of 2016 reported similar proportion of lifetime use as 2013, 43% and 42% respectively. However, it was also noted that there has been a gradual increase since 2001, which reported 38% of the Australian population aged 14 or older had used illicit drugs at some point of their life\textsuperscript{3}. Cocaine, synthetic cannabinoids and psychoactive substances all demonstrate an increase in proportion of lifetime use, namely from 8.1% to 9.0%, from 1.3% to 2.8% and 0.4% to 1.0% respectively\textsuperscript{3}. Approximately 15.6% of the Australian population aged 14 or older have reported illicit drug use within the last 12 months in 2016 and has been stable since 2004\textsuperscript{3}. There was no significant increase in any specific illicit drug use since 2013\textsuperscript{3}. In relation to the use of novel psychoactive substances (NPS), the NDSHS reported an increase of more than doubling, from 1.3% to 2.8%, of lifetime use of synthetic cannabinoids but a decrease in recent use from, 1.2% to 0.3%, from 2013 to 2016. Simply
meaning that there was an increase of experimentation among the population but a reduction in chronic use.

The impact of illicit drug use on society can be classed into two types: tangible and intangible costs. Tangible costs are the quantifiable costs to society, which can be categorised as those resulting from (1) workplace labour, (2) labour in the household, (3) healthcare and (4) crime. Examples of the quantifiable costs as a consequence of illicit drug use from each category are briefly elaborated; (1) reduction in the workforce and absenteeism, (2) premature death and illnesses, (3) pharmaceuticals, hospital and medical costs, and (4) policing, courts and prison.

In 2004-05, these costs totalled to just over 6.9 billion dollars, combining the tangible costs from workplace labour, household, healthcare and crime. This makes up 0.88 percent of the gross domestic product. Intangible costs refer to unquantifiable costs from, for example, the loss of life or loss of productivity in a workplace as a consequence of illicit drug use. The intangible cost attributed to loss of life and pain and suffering were almost 1.3 billion dollars.

Burden can be qualitatively expressed as years of lost life (YLL) or years left with disability (YLD). In 2018, the Australian Institute of Health and Welfare reported that illicit drug use was responsible for 2.3% of the total burden of disease and injury in Australia for 2011 and 1.3% of deaths. Years of life lost (YLL) as a result of illicit drug use was equivalent to 70,419, 3.1% of all fatal burdens in Australia, and of the non-fatal burdens, illicit drug use was also responsible for 1.4% of the total, equivalent to 31,447 years lived with disability (YLD). It was also reported that lower socio-economic groups contribute the greatest burden as a result of drug use. In 2004/05, the cost of crimes that could be attributed to illicit drugs was found to total almost 4 billion dollars, with 43% of costs contributed by the police sector alone.
same period, net cost impact on healthcare resulting from illicit drug use was reported to total 201.7 million dollars\(^4\). The collective impact of illicit drug abuse on the state and federal budget in 2004/05 was reported to total to almost 2.4 billion dollars\(^4\). It is important to note that this sum does not include the budgetary impact of illicit drug use, but solely its abuse. Between 1998/99 and 2004/05, there has been an 11.3% increase in total cost of illicit drug use; at 2004/05 prices\(^4\). It is important to understand that the culture of illicit drug use has drastically changed, with the decline in the prevalence of some illicit drug use and the emergence of new drugs such as NPSs.

NPS are synthetic substances that are made to mimic the effects of existing psychoactive drugs such as cannabis, ecstasy (MDMA), heroin and cocaine\(^5,7\). The European Drug Monitoring Centre for Drugs and Drug Addiction reports that since 2010, there has been a significant increase in the first detection of new NPS, with 70% of all being detected in the last 5 years alone\(^7\). The production of these NPSs occurs at a faster rate than the ability to assess the possible detrimental effects of the drug. How this occurs, is that production of substances that mimic already existing substances do not require a lot of work. It involves minor changes in the chemical structure of the substance that allow it to be undetectable to current screening methods for the existing substances. It is for this reason that most NPSs avoid legislation and appear.

In the past, the methods of measuring societal drug use were dependent on community and government surveys\(^3,8-12\). Surveys are often open to self-report bias, as they are reliant on an individual’s recollection of events. This problem could be compounded when examining the prevalence of illicit drug use, as the physiological and psychological effects of drugs could
further impact recollection. For example, the effects of cannabis use include polyphagia, colloquially known as “the munchies”, euphoria, and impaired short term memory\textsuperscript{13,14}; or the use of cocaine elicits paranoia, mydriasis and extreme elation and energy\textsuperscript{15-17}. It is recognised in law that the actions and memory of an individual while under the influence would often result in the discreditation of their statement in court\textsuperscript{18}. To this effect, then, the reliability of the individual to recollect the events of drug use becomes, furthermore, unreliable.

The standard method of collecting community data is through conducting surveys. Often in paper form, they can also be conducted via the phone or electronically. The cost of distributing and conducting surveys, and then additionally analysing them thereafter is costly\textsuperscript{19}. A study assessed the voluntary participation in a weekly survey regarding illicit drug use; found that within a population of 29,083, only 1% of individuals completed the surveys\textsuperscript{20}. In addition to conducting surveys, data on drug use can be gathered from police, medical reports and workplace drug testing. The National Drug Strategy Household Survey is an Australian based voluntary questionnaire that has been conducted every 2-3 years since 1985, collecting information on the use and perception of alcohol, tobacco, illicit drug and pharmaceuticals\textsuperscript{3,8-12}. The most recent NDSHS conducted in 2016 is the first in the series to introduce the option of completing the form online\textsuperscript{3}. In this instance, the mode in which the survey was conducted may have had an impact on the responses given. For example, the persons completing the survey may have responded in the way they think the interviewer wants them to respond or may deter from revealing information in some cases where the action is illegal. The 2016 NDSHS did establish an increase in response rate from 49.1% and 50.6% in 2013 and 2010 respectively to 51.5%. This increase could possibly be attributed to the introduction and ease of completion of the online form option, creating incentive to
continue online forms. However, the increase is slight and may have been attributed to other unrelated factors³.

In order to accurately portray the level of societal drug use, data must be presented in an unbiased and objective manner. Wastewater-based drug epidemiology (WBDE) has been proposed as a method that can objectively and directly measure societal drug use²¹. Wastewater-based epidemiology (WBE) is the analysis of wastewater (WW) to understand the incidence and distribution of various factors relating to health. WBDE to estimate societal drug use is essentially the analysis of WW to detect drug analytes, which are then utilised to evaluate real-time societal drug use based on the population the WWTP serves²⁰-³⁹. The concept was first proposed by Daughton et al. with the aim to assess the effect of drug excretion products on the environment, specifically aquatic life²¹. Since then, the theory has been broadened to assess the extent of societal drug use. A study conducted by Jones-Lepp et al. was the first to demonstrate a method to detect and confirm the presence of illicit drugs in effluent WW⁴⁰. Zuccato et al. laid out the first initial studies contributing to WBDE to estimate societal drug use and assessed cocaine use in Italy by back-calculating using the four factors, drug concentration detected, water flow rate, excretion rate of the drug target residue (DTR) and the number of people served by the WWTP²⁷. Since then, many studies have adapted this method to optimise it from a robust method to a method that can deliver with greater accuracy the knowledge of societal drug use. Wastewater-based drug epidemiology studies are still in their infancy and demonstrates great potential in their ability to estimate societal drug use. WBDE has the potential to track and relay near real-time community drug use and portray patterns of use throughout a time course: over the course of the week, year or public holidays²⁰-²⁶,²⁹,³¹-³³,³⁷-³⁹,⁴¹-⁴⁹; or during the time leading towards a
large public event\textsuperscript{50}, as well as the pattern of use between drugs. It can identify hotspots of drug use to assist policy makers and law enforcement to better target their efforts. An added advantage WBDE has over other methods of data collection on drug use in a community, such as those gathered from surveys, police and medical reports, is that the methods of WBDE are non-invasive and retain the anonymity of drug users. The data collected from these studies have no way of being traced back to any singular individual and therefore will not implicate them in any illegal activity of illicit drug use. The aim of WBDE is not to incriminate individuals but to provide accurate near real-time information on illicit drug use in the community to the relevant parties. For these reasons, the methods of WBDE require validation so that they may successfully and efficiently be utilised. This critical review aims to assess the validity of the currently available methods, in their ability to objectively and directly evaluate drug levels in WW to estimate societal drug use.

5.1 THE PURPOSE OF MEASURING SOCIETAL DRUG USE

The understanding of true societal drug use is highly important. With the ability to accurately assess real-time societal drug use, the efforts of various community groups such as law enforcement, medical professionals and the government bodies and policy agencies, can be better directed and budgeted. Drug use has a large impact on every facet that makes up a community. Millions of individuals have reported to be current users of illicit drugs such as cocaine, heroin, amphetamine and methamphetamine\textsuperscript{3}. The consequences of the use of these drugs produces a negative ripple effect to the rest of the community. From eliciting significant negative consequences on individual health, to diminishing social behaviour, extending its impact to family and friends, and then to the rest of the community. The impact of illicit drug use is devastating, with some individuals developing substance abuse disorder,
addiction. Estimates on societal drug use could help highlight and raise awareness to the drug epidemic. And by understanding the different levels of various illicit drug use in society, allowing the best strategies to be formed to combat them.

Addiction is a devastating consequence of drug use. In 2015-16, the number of treatment episodes conducted for alcohol and drug treatment had a 40% increase from 140,475 to 198,747 episodes, with treatment for drug use accounting for the majority over the past 10 years. Drug clinics and rehabilitation centres offer different detoxification and treatment programs depending on the illicit drug that has caused the addiction. No one medication program assists with all drug addictions. Each program requires various concoctions, various doses and dosing schedule and plans. By understanding the level of drug use in society, this may allow drug clinics and rehabilitation centres effectively organise, budget and regulate the pharmaceuticals required for each program.

Though drug use may have gradually decreased throughout the years, there has been an emergence of more potent, dangerous drugs. New psychoactive synthetic drugs are an emerging group of drugs coming into circulation. As technology advances, new synthetic drugs come into play requiring better screening techniques to adequately evaluate its contribution to drug use in the community. Generally, the basic screening methods only search for chemicals that have specifically been targeted. Newer screening methods would aim to identify new substances without advertently targeting specific substances.
With better developed and refined techniques, WBDE could potentially identify trends in drug use that can then be used to devise programs or campaigns to best combat the use of dangerous illicit drugs that harm the community.

6. DRUG PHARMACOLOGY

Drugs can be described as substances that produce a physiological or biochemical effect on the body. Each drug has a different potency, which requires different doses in order to elicit the same level of effect to another drug as well as dictate the route of administration to obtain optimum effects of the drug. This is also affected by the individual’s age, ethnicity, genetic dispositions, weight and the presence of other diseases and the concurrent use of other drugs. Though drugs will produce characteristic symptoms and effects, it is also important to note that each individual may exhibit slightly different reactions to a certain drug compared to another individual.

Pharmacology is the study concerned with the nature, effects and mode of action of drugs on our body. Underpinning the study of pharmacology are the two broadly characterised phases of a drug’s effect: pharmacodynamics and pharmacokinetics. Pharmacodynamics describes the effect a substance will have on the body, whereas pharmacokinetics describes the body’s actions onto the drug. Understanding the pharmacology of substances is critical in everyday life, and it would, therefore, would be appropriate to incorporate it into WBDE.

6.1 PHARMACODYNAMICS

Pharmacodynamics is the study of the magnitude of drug response on the body. Specifically, it refers to the binding of the drug to the drug target and the response produced from the
binding, the intensity and duration of drug response and its relation to drug concentration\textsuperscript{55,56}. Drug response occurs once the drug binds to its corresponding receptor and produces a chemical reaction by altering the conformational arrangement of the receptor\textsuperscript{55,56,59}. At high drug concentrations, more receptors are occupied resulting in a higher response than low drug concentrations where fewer receptors would be occupied\textsuperscript{55}. The type of stimulus produced by the drug depends on the relationship between the drug and the receptor, whether the drug is an agonist, partial agonist or antagonist\textsuperscript{59}. An agonist would mimic the response produced by the receptor-ligand when it binds to the receptor, whereas when an antagonist binds to its receptor, it would inhibit and block the response usually produced by the binding of a receptor ligand\textsuperscript{55,56,59}. And a partial agonist would elicit a fraction of the response produced by a receptor ligand binding\textsuperscript{55,56}. Drug efficacy and affinity with the respective receptor are factors that also influence the overall response produced by the drug\textsuperscript{55}. Typically, the interaction between the receptor and the corresponding drug is reversible in nature and once drug response is completed, the drug dissociates from the receptor and drug response dissipates. The events that occur to the drug after this is referred to the pharmacokinetics of the drug\textsuperscript{56,60}.

6.2 PHARMACOKINETICS

Pharmacokinetics describes the study of drug concentration in relation to time and movement in the body, from administration, distribution throughout the body and excretion from the body\textsuperscript{55,56,60}. Therefore, it is the study of the absorption, distribution, metabolism and elimination (ADME) of the drug and how these aspects affect drug concentration in the body\textsuperscript{55,58}. Drug concentration cannot be measured directly, instead plasma concentration is
measured instead\textsuperscript{56}. Plasma concentration is assumed to reflect true drug concentration with often a linear relationship, however some drugs may exhibit greater, complex relationships\textsuperscript{55}.

\textbf{6.2.1 ABSORPTION}

Common routes of administration include oral, topical, intravenous and intraperitoneal injections as well as nasal delivery, especially in regards to the intake of illicit drugs\textsuperscript{29,55,58}. Absorption refers to the uptake of the drug into systemic circulation and is dependent on its various properties, dictating the drug’s ability to be readily absorbed through membranes\textsuperscript{55,56,58,60}. Drugs usually pass through membranes by means of passive diffusion, however some uptake transporters may assist in facilitating the movement\textsuperscript{58}. Route of administration affects the rate in which the drug is absorbed into the systemic circulation. For example, an oral administration would take more time than an intravenous administration as the drug must travel to the gastrointestinal tract before dissolving, and passing the membrane before entering the systemic circulation, whereas the latter would deliver the drug directly into the systemic circulation\textsuperscript{55}. Circumstances may also dictate the route of administration, whether the individual requires or desires immediate and maximum effect of the drug. The form in which the drug comes also impacts the rate it enters the systemic circulation as well as the magnitude of the response\textsuperscript{59,61}. Common forms include tablet, capsule, powder, syrups, in a solution or pure liquid form. In conjunction with these aspects, the chemical properties of the drug, whether it is lipid soluble, aqueous soluble, polar or non-polar in nature, collectively impacts the rate and ability of drug absorption into the systemic circulation and the final drug concentration.
2.2.1.1 BIOAVAILABILITY

Bioavailability of the drug is a common aspect in understanding the pharmacokinetics of the drug as it describes the fraction of the dose that successfully enters the systemic circulation\textsuperscript{55,56,58,61}. It can be further divided into three categories; fraction absorbed, intestinal bioavailability and hepatic bioavailability\textsuperscript{56}. Portions of the drug can be lost at each of these points, and so collectively these factors together will describe overall bioavailability of the drug\textsuperscript{56}.

6.2.2 DISTRIBUTION

The process of distribution of the drug within the body is concerned with its ability to access its site of action with the target receptors and the relative distribution between plasma and the rest of the body\textsuperscript{55,58}. The rate and extent at which the drug distributes within the body is important to evaluate plasma drug concentration since direct tissue concentration cannot be reasonably measured\textsuperscript{56}. The extent to which a drug has distributed to plasma relative to tissues assists in the understanding of the drug availability for the magnitude of drug response. Extensive distribution to the plasma compared to tissue would mean a lower magnitude of response as a large fraction of the drug is delivered to certain organs for elimination. Conversely, extensive distribution to tissues would enhance drug response but impede elimination, possibly leading to toxicity due to excess accumulation of the drug in the body\textsuperscript{56}. Distribution is driven primarily by passive diffusion based on the concentration gradient of the unbound drug\textsuperscript{55,56}. The drug may bind to plasma proteins or tissue macromolecules, which cannot participate in the concentration gradient\textsuperscript{56}. This impacts the diffusion of the unbound drug. At equilibrium, the concentration gradient for the unbound drug is no longer a driving mechanism for diffusion, however a drug that extensively
distributes in plasma and binds to plasma proteins will contribute to a greater overall concentration of the drug in plasma\textsuperscript{55,56}. The binding of the drug to plasma proteins or tissue macromolecules is an important factor in understanding the distribution pattern of the drug, dictating the areas in which the drug has access to\textsuperscript{55,56,58}.

### 6.2.3 METABOLISM

Drug metabolism is the conversion of the parent drug to metabolites preparing it for removal from the body\textsuperscript{55,56,58,61}. In some cases, the metabolism of the parent drug releases the active metabolite. These drugs are termed ‘pro-drugs’ and are chemically manufactured that way to ensure that the drug is not metabolised too rapidly before it is able to elicit its response\textsuperscript{58}. The metabolite is then eliminated via the renal or biliary route\textsuperscript{55,58}. The metabolic processes of metabolism in the liver are categorised as either a phase I or phase II process\textsuperscript{56}. Phase I involves small modifications to the parent drug such as the removal of a methyl group or the addition of a hydroxyl group, both oxidation and reduction reactions may occur. Phase II processes describe the conjugation of the parent drug or the resultant phase I metabolite with a polar molecule via UDP-glucuronosyltransferase (UGTs) enzymes\textsuperscript{55,56,58,59}. The liver houses the largest number and variety of drug-metabolising enzymes. The most important family of enzymes are the cytochrome P450 enzymes which are responsible for approximately 75\% of drug metabolism processes\textsuperscript{58}.

### 6.2.4 ELIMINATION

The rate at which the drug undergoes metabolism and elimination is described by the elimination rate constant and half-life of the drug\textsuperscript{56}. Factors that affect this are the determined by two factors, clearance and volume of distribution\textsuperscript{55,56,58,61}. Clearance is a
constant that describes the ability of the elimination organs to efficiently extract the parent drug from the systemic circulation and eliminate it from the body\textsuperscript{61}. It is essentially the proportionality between the rate of elimination and the present drug concentration in the plasma\textsuperscript{61}. Volume of distribution describes the relative drug concentrations diffused between the plasma and tissue\textsuperscript{55,58,61}. In order for the liver and/or kidney to have the opportunity to metabolise the parent drug and remove it from the body, it must have access to it by being present in plasma\textsuperscript{56}. It is important to note that these primary parameters are independent of each other, and changes to either one does not affect the other parameter\textsuperscript{56}. Total body clearance is additive of renal, hepatic and any other form of clearances\textsuperscript{56}.

6.3 PHARMACOEPIDEMIOLOGY

Pharmacoepidemiology is the study of the distribution of drug effect within a population. Hennessy discusses the pharmacological aspects that contribute to pharmacoepidemiology studies\textsuperscript{55,57}. Genetic polymorphisms are an example of population variation to pharmacodynamics of drug effect\textsuperscript{55,57}. Age, diseased states, adapted drug responses and drug-drug interactions are other factors discussed by Hennessy that impact the pharmacodynamic drug response\textsuperscript{57}. In addition, there are pharmacokinetic factors that are also considered in pharmacoepidemiology studies. Pharmacokinetic factors such as absorption, distribution, metabolism and elimination of the drug are also affected by genetic variabilities, age, diseased adaptive drug responses and drug-drug interaction\textsuperscript{57}. Metabolism of drugs can either inactivate the parent drug or convert an inactive pro-drug to its active counterpart which will produce a therapeutic effect\textsuperscript{58}. This process is assisted by the action of enzymes. Enzymes are proteins that are encoded for in DNA. For example, cytochrome P450 (CYP P450) is a major enzyme class that is responsible for almost 80% of all drug
metabolism processes in the body\textsuperscript{62-64}. The genetic coding of these enzymes has been found to exhibit variations between individuals within population, as well as between ethnicities that are present within a population\textsuperscript{62-65}. Extensive studies have been dedicated to the genetic variations that occur within the population with the expression of the CYP P450 enzymes\textsuperscript{62-64,66,67}. A characteristic that has been able to be determined from these studies is the categorisation of certain CYP P450 enzyme generating the phenotypes; ultra-metabolisers (UM), extensive metabolisers (EM), intermediate metabolisers (IM) and poor metabolisers (PM)\textsuperscript{66} of the corresponding drugs metabolised by the specific CYP P450 enzyme. Therefore, the rate and extent a drug is metabolised will also influence the level of excretion from the body. As demonstrated, the understanding of pharmacoepidemiology of the population in question can contribute to WBDE to aid in obtaining accurate societal drug estimates tailored for that population.

7. DRUG ABUSE

Illicit drug use has become an epidemic throughout many societies, casting burdens on the healthcare, society, law enforcement and the government. The total burden from illicit drug use has been reported to have increased by 6.9\% from 2003 to 2011 in the latest report from Australian Institute of Health and Welfare on the impact that alcohol, tobacco and illicit drugs on the burden of disease\textsuperscript{6}. They also report that illicit drug dependence was 5.6\% higher in 2011 compared to 2003. Many government reports and scientific studies directed towards illicit drug use have found that cocaine, methamphetamine, amphetamine, ecstasy, heroin and marijuana, to name a few, are typically the drugs with high abuse rates\textsuperscript{68-71}. An emerging class of drugs of abuse are the NPSs, which are designed and created to mimic the physiological and psychological effects of existing psychoactive drugs\textsuperscript{5,7,41}. These are
particularly significant, as many are not covered under current legislation, making them technically ‘legal’ for use. The use of these illicit drugs bring about a sense of euphoria, relaxation and a sense of escape from their everyday lives, which makes drug use appealing to these individuals\textsuperscript{13,59}. Side effects of a drug is defined as any undesirable physiological or psychological implications, and the risk of side effects accompany any type of drug use. These may include paranoia, irritability, explosive violent behaviour and indifference to pain\textsuperscript{13,17,58,61}. A genuine risk that accompanies continuous drug abuse is the possibility of becoming addicted to the substance. Drug addiction is defined as the chronic disease that is characterised by compulsive actions to seek out the drug and obtain it by any means\textsuperscript{72}. It is defined as a disease due to the changes that occur to the brain chemistry and structure that occur due to the repeated drug abuse\textsuperscript{73,74}.

8. WASTEWATER TREATMENT PLANT

Wastewater encompasses all the water that has been altered by human use, either within a household, corporation, or commercial setting\textsuperscript{75,76}. This includes used water from toilets, sinks, manufacturing processes, shops and stormwater\textsuperscript{76,77}. WW is collected and pooled within sewage pipes and transported to WWTPs where it undergoes a series of treatments to remove waste so that the water may be released back to the environment\textsuperscript{75-77}. Initially, the primary treatment involves the removal of solid materials, including plastics and smaller particles such as sand\textsuperscript{77}. The resultant WW then flows through large tanks where solids settle at the bottom of the tank and removed as sludge and oils and grease on the surface are skimmed\textsuperscript{77}. Subsequently, secondary treatment utilises micro-organisms and aeration with oxygen to breakdown remaining wastes and fine particles and organic pollutants respectively\textsuperscript{77}. WW sampling for experimental methods to estimate societal drug use can
either occur ahead of the treatments WW usually undergoes or subsequent to the treatments\textsuperscript{21}. WW before treatment is termed influent water, and subsequent to the treatments is termed effluent water\textsuperscript{21}.

WW sample collection for WBE can either be collected as influent or effluent WW. Collection of influent water for samples mean that no treatment has been applied and methods would require a filtration and isolation of the drug metabolites from the contaminants\textsuperscript{21}. Using effluent WW samples should be done with caution, as wastewater treatment processes could potentially remove some of the drug metabolites that are the target for WBDE\textsuperscript{21}.

WBDE is a relatively new area of research that can be utilised to estimate societal drug use. It involves the measurement of drug analytes, which can be in the form of drug metabolites, parent drug or drug biomarkers, in WW samples\textsuperscript{21,27}. By incorporating the flow rate and the population number that the WWTP serves, a back-calculation can be performed to estimate the level of illicit drug use within the community that the WWTP serves\textsuperscript{27}. WBDE hinges on the understanding that consumed illicit drugs are eventually excreted from the human body.
in one form or another, often as a major metabolite in urine\textsuperscript{21}. These drug analytes are the DTRs for WW analysis.

9. WASTEWATER-BASED DRUG EPIDEMIOLOGY

Wastewater-based epidemiology (WBE) is the area of study that is concerned with the analysis of WW to understand the incidence and distribution of factors relating to the health and welfare of the community. Wastewater-based drug epidemiology (WBDE) is specific to the distribution and incidence of drug use and the impact it poses to the welfare and health of the community. The theory behind WBDE is the understanding that when a drug is consumed, it is metabolised and broken down in the body and eventually eliminated in urine. It is the metabolites and biomarkers of the drug that are eliminated in urine that may be detected in WW\textsuperscript{21}. From this, the drug concentration in WW can be determined and furthermore this can then be used to back-calculate the level of drug use in the community\textsuperscript{21,27}. It has the benefit in that the methods involved in WBDE analysis are non-invasive, with the potential to provide an estimate of societal drug use without implicating individuals within the community in the incriminating act, which has often been a deterrent when completing voluntary surveys directed to recent drug use. WBE has also been utilised to measure the use and misuse of pharmaceuticals, specifically to assess the impact of excreted chemicals in WW to the environment and aquatic life\textsuperscript{21}. The data collected from these studies can be utilised to illustrate the level of drug use in society, for example, which drugs have the highest rate of abuse and highlight various trends that can occur throughout the week or leading up to and around large public events such as music festivals. WBE for societal drug use can be coupled with other studies to assess the impact of excreted illicit drugs to the environment\textsuperscript{21}. Research has been put forward to assess the use of legal
pharmaceuticals, but lack in the understanding of the impact that illicit drug use has on the surrounding environment\textsuperscript{21,78,79}. Unlike surveys, the information collected from WBDE can provide close to real-time information on societal drug use with preliminary findings available much faster than collating all the information gathered from surveys. Surveys generally have a high rate of non-response,\textsuperscript{20} which is overcome with WBDE. WW sample collection can combine the entire population that the treatment plant serves and provide an estimate of societal drug use. Surveys rely on adequately sampling a normally distributed population as it requires extrapolation to encompass and include the population that did not partake in the voluntary survey. By doing so, estimates can involve large error rates and significantly misrepresent the level of illicit drug use in the community, even more so where surveys obtain little response rate\textsuperscript{5,20,21}. Information on illicit drug use also comes from the arrest and drug seizure reports from law enforcement. However, this information provides a significant bias in that virtually all the drug data is from illegal and incriminating acts\textsuperscript{21}. Societal drug use can also be provided by healthcare and medical centres, like hospitals and general practices, but such information may be biased due to doctor-patient confidentiality\textsuperscript{21}. Additionally, again, illicit drug users can be wary of approaching professionals in fear of incriminating themselves and being persecuted for the participation in an illegal act\textsuperscript{3}. Over time there has been a decrease in the use of any one type of an illicit drug, but also saw an increase in the use of another, ecstasy or designer drugs\textsuperscript{4} (Table 1).

Therefore, there is a need to have the ability to track trends in drug use. Using WBDE, trends and hotspots in illicit drug use can be identified and used as a means to devise and assess the impact of policies or strategies that can effectively target and combat the drug use epidemic. However, data collected from WBDE on societal drug use cannot be depended upon alone to
measure the success or non-success of drug policies and strategies, rather incorporated into a framework that can assess this. Although WBDE studies are still in their infancy, majority of which have been conducted within the last decade, they have demonstrated a great potential in understanding and obtaining an objective and unbiased estimate of drug use in a community.

Table 1. Prevalence of drug use within a population aged 14 and older from 1998 and 2004, and 2004 as a function of 1998 drug use rates

<table>
<thead>
<tr>
<th>Drug</th>
<th>1998 per cent</th>
<th>2004 per cent</th>
<th>2004 as per cent of 1998</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marijuana/cannabis</td>
<td>17.9</td>
<td>11.3</td>
<td>63</td>
</tr>
<tr>
<td>Steroids</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Barbiturates</td>
<td>0.3</td>
<td>0.2</td>
<td>67</td>
</tr>
<tr>
<td>Inhalants</td>
<td>0.9</td>
<td>0.4</td>
<td>44</td>
</tr>
<tr>
<td>Heroin</td>
<td>0.8</td>
<td>0.2</td>
<td>25</td>
</tr>
<tr>
<td>Methadone</td>
<td>0.2</td>
<td>0.1</td>
<td>50</td>
</tr>
<tr>
<td>Other opiates/opioids</td>
<td>n.a</td>
<td>0.2</td>
<td>n.a</td>
</tr>
<tr>
<td>Meth/amphetamine</td>
<td>3.7</td>
<td>3.2</td>
<td>86</td>
</tr>
<tr>
<td>Cocaine</td>
<td>1.4</td>
<td>1.0</td>
<td>71</td>
</tr>
<tr>
<td>Hallucinogens</td>
<td>3.0</td>
<td>0.7</td>
<td>23</td>
</tr>
<tr>
<td>Ecstasy/designer drugs</td>
<td>2.4</td>
<td>3.4</td>
<td>142</td>
</tr>
<tr>
<td>Ketamine</td>
<td>n.a</td>
<td>0.3</td>
<td>n.a</td>
</tr>
<tr>
<td>GHB</td>
<td>n.a</td>
<td>0.1</td>
<td>n.a</td>
</tr>
<tr>
<td>Injected illegal drugs</td>
<td>0.8</td>
<td>0.4</td>
<td>50</td>
</tr>
<tr>
<td>Any illicit drug</td>
<td>22.0</td>
<td>15.3</td>
<td>70</td>
</tr>
</tbody>
</table>

9.1 THE FOUR PARAMETERS

There are four typical parameters required for WBDE studies: 1) concentration of the drug residue target found in WW, 2) flow rate at the WWTP, 3) excretion factor of the drug residue target and 4) the number of people the treatment plant serves. Each of these factors requires accurate methods of acquiring their respective data. Zuccato et al. laid out the first initial studies relating to WBDE to estimate societal drug use by utilising the four factors mentioned previously to back-calculate the level of illicit drug use within a community. The basic foundations of the study involved solid phase extraction (SPE) techniques to extract drug residue targets present in samples collected from influent WW, together with quantitation methods utilising LC-MS/MS to determine drug concentration. Secondly, flow rate data of
Through the treatment plant are often acquired directly from the treatment plant at which the sampling process is conducted. Thirdly, excretion factors of the drug residue targets are obtained from previous literature on the pharmacokinetics of the drug. And finally, the number of people that the WWTP serves is estimated from census surveys conducted by the government. Since the utilisation of WBE to estimate societal drug use, each of these factors has undergone refinement as technology advances and more studies are contributing to this new area of research.

9.1.1 DRUG CONCENTRATION FROM DRUG ANALYTES

The quantitation of DTRs with LC-MS/MS is an important step in the determination of societal drug use. It serves as one of the parameters in the back-calculation for the estimate of illicit drug use in the community. For this reason, there is a high importance placed on the selection of DTR, its collection from WW, storage and extraction, since they collectively, they impact the accuracy of this parameter. Linked to this parameter, is the knowledge of the selected DTRs’ excretory factors. Specifically, the fraction DTR is excreted from the parent drug. Additionally, results may often be presented as doses/day/1,000 people, which requires the knowledge of the typical dose of the drug, and subsequently the fraction of the dose that is excreted as the DTR.
9.1.1.1 SAMPLING COLLECTION, PREPARATION AND STORAGE

The very first step in WBDE is the collection of WW sample. WW samples were generally collected as 24 hour composite samples of influent water. Sampling influent or effluent WW is dependent on the aim of the study. Utilising WBE to estimate societal drug use would be most beneficial from collecting influent WW samples, as sampling would occur before WW undergoes treatment, eliminating the risk of the DTR being removed before they are analytically detected through the study. Collection of effluent water would better suit the aim to assess the effectiveness of WWTPs in removing illicit drug residues from the water, and furthermore the impact those residues have on the aquatic life and agriculture once they are pumped back into the environment. Initially, samples were collected by an automatic sampling device and kept refrigerated at 4 °C on site during the 24-hour collection, before analytical samples were derived from the composite samples. A composite sample describes a whole sample composed of subsamples collected across a selected population and/or time-frame at specific intervals with the intention of obtaining a representative collective sample. In this case, for WBDE studies, 24-hour composite samples are collected to represent the total drug levels of a population for the day the samples are collected. Automatic sampling devices were either time-proportional, which collected a certain volume of WW at the allocated time intervals, or volume- or flow- proportional which collected either collects a certain volume of WW at time intervals that is determined by the flow of WW or a variable volume of WW is collected at defined time intervals. A study conducted by Castiglioni et al. assessed the uncertainties that are involved in WBDE. A comprehensive questionnaire was developed in order to systematically assess the sampling methods of various WWTPs in the study. They had concluded that a volume- or flow-proportional automatic sampling device would minimise the relative standard deviation involved with sample collection, as long as it was
performed in conjunction with short sampling intervals\textsuperscript{84}. Alternatively, polar organic chemical integrative sampler, POCIS, has been demonstrated in the recovery of some illicit drug analytes present in effluent water\textsuperscript{40}. However, there are several limitations in its utilisation. Firstly, only drug residue targets that are polar in nature are collected by this specific sampler, and so there is a risk of some DTRs being missed by this device. This will result in results misrepresenting societal drug use. Samples are taken from the raw WW composite on site once the 24-hour composite sample has been collected. Best practice sampling methods were recommended and assessed for their ability to represent daily composite samples with minimal uncertainty by Castiglioni et al\textsuperscript{84}. Briefly, the sampling protocol provided to the staff at the WWTP were as follows; a 400 mL subsample would be aliquoted from the 24 hour composite influent WW collection into a polypropylene bottle that has been pre-rinsed with Milli-Q water and methanol, the sample is then acidified on-site at pH 2, using 2.5 mL of hydrochloric acid solution\textsuperscript{25,84}. Acidification of the sample would reduce the likelihood of in-sample transformations of the drug residue targets\textsuperscript{85}. The samples are stored at -20 °C in the dark until chemical analyses are conducted within 2 months. Some studies have opted for other methods of storage depending on the schedule of analytical testing, such as storage at 4 °C in the dark, if the sample is to be analysed within 3 days.

\textbf{9.1.1.2 SELECTION OF DRUG TARGET RESIDUES (DTR)}

At this point, WBDE for estimating societal drug use is only able to screen and evaluate known illicit drugs. This is because the excretion factors need to be known in order to be able to back-calculate to obtain an estimate of societal drug use for that illicit drug. Alternatively, if a suitable DTR is identified, it may be utilised merely to detect the presence of that illicit drug. Studies are often conducted to select and identify suitable DTRs. DTRs identified tend to be
metabolites resulting from the internal metabolism of the parent drug so that estimated drug use from WBDE can accurately demonstrate drug abuse resulting from human use. Selecting an appropriate DTR is important to establish a foundation for the remainder of WBDE, especially for the back-calculation that estimates the level of illicit drug use of the community. The selected DTR must fulfill specific criteria. The DTR should be stable in WW\textsuperscript{26,85-89}, a significant excretion product\textsuperscript{26}, have sufficient data on their excretion factors\textsuperscript{26,29} and have a chemical profile that has a reasonable recovery rate from extraction methods\textsuperscript{90}, in order to accurately extrapolate to estimate societal drug use.

A study conducted by Castiglioni et al. evaluated cocaine and its metabolites as potential DTRs in WW, by assessing each of their stabilities in WW as well as the recovery rate based on the gold standard methods of SPE followed by the analysis with (LC-MS/MS)\textsuperscript{90}. In cases where the chemical profile of the potential DTR under assessment did not allow for a good recovery rate from SPE, steps can be taken to optimise the retention of the metabolite analyte during extraction methods. For example, ecgonine, a minor metabolite assessed as a potential DTR for cocaine, is highly polar and water-soluble resulting in a weak recovery rate from SPE using Oasis MCX cartridges (60 mg)\textsuperscript{90}. More sorbent material (150 mg) was added to the cartridges in order to overcome this successfully increasing the retention of ECG on Oasis MCX cartridges\textsuperscript{34}. Studies conducted in WBDE to estimate societal drug use have used the main major excretory metabolite as the DTR and often include in-study validation of the methods conducted. Although these tend to be the ideal DTR for these studies, limited studies have been dedicated to assessing their ability to directly portray illicit drug use of the parent drug. The pharmacology of drug action is very complex. The pharmacokinetics of the drug is influenced by many factors, as previously discussed, including drug-drug interactions (DDI).
These can lead to side effects and is often the reason why some medication cannot be taken in conjunction with another. A study conducted by Harris et al. described the variation in the pharmacology of cocaethylene, a metabolite of cocaine, following the concurrent consumption with alcohol. This was noted in a study conducted by van Nujis et al., and a correction factor was implemented in their study on WBDE focused on estimating cocaine use within Brussels, Belgium\(^2\). It has been demonstrated then, that drug excretion profiles are impacted by substances taken in conjunction to the drug, and therefore studies have the need to incorporate uncertainties regarding drug excretion profiles in combination with other common substances.

**9.1.1.3 SAMPLE EXTRACTION AND CHEMICAL ANALYSIS**

A vast number of studies have continuously utilised SPE to extract and isolate drug residue targets from the samples collected from WW\(^{22,24,26,27,36,44,46,89,91,92}\). Some studies have also opted for a direct injection method, which has been tested to reduce DTR degradation\(^{38,88,93}\). However, storage methods that have been mentioned above, also have been utilised as a way to reduce degradation of DTR in samples before analysis can be performed. Wastewater based epidemiology to estimate societal drug use often underestimates the level of drug use within a community. It has been suggested that this is partly due to the disregard of the sorption of DTR) into suspended particulate matter (SPM) which is usually filtered from the sample\(^{32,94,95}\). To overcome this, an additional extraction method, pressurised liquid extraction (PLE), have been incorporated into WBDE. However, there is also conflicting evidence that the absorption of illicit drug analytes into SPM, which is filtered prior to SPE methods, does not result in significant underestimation of WW illicit drug concentration\(^{46}\).
Samples are spiked with internal standards to serve as quality control and assess instrument performance and the precision of the quantitative analysis. Overwhelmingly, LC-MS/MS has remained the gold standard to identify and quantify the levels of DTRs in samples. Specifically, high-performance liquid chromatography (HPLC) coupled to a triple quadrupole (QqQ) mass spectrometer equipped with electrospray ionisation (ESI) interface. Electrospray ionisation is the technique of choice to produce ions for LC-MS/MS. Multiple reaction monitoring (MRM) mode was often favoured to perform the quantitative analyses, as it has the capability to assess multiple product ions from one or more precursor ions. LC-MS/MS is highly recognised and accepted within the field of WBDE, but an ongoing limitation with this method is that LC-MS/MS is only capable of quantifying and analysing targeted drug residues for which this technique has been developed for. Those that are not actively screened for, tend to be omitted and ignored from studies. To overcome this, high-resolution MS can be incorporated as it demonstrates the potential to screen for non-targeted drug residues\textsuperscript{41,96-98}. A recent Australian study has also evaluated the use of a data-independent acquisition (DIA) method of Sequential Window Acquisition of all THeoretical fragment-ion spectra, most commonly referred to as SWATH, concurrent to liquid chromatography-quadruple time of flight instrument to qualitatively screen for compounds in WW\textsuperscript{39}. Utilising a continuously updated database, SWATH was able to identify and confirm the presence of 100 compounds within WW, with 61 compounds confirmed to be present within all samples collected across the 14-month period\textsuperscript{39}. This study was able to identify the use of two new psychoactive substances in South Australian WW, subsequently quantified by high-performance liquid chromatography coupled with a triple, quadrupole mass spectrometer instrument equipped with electrospray ionisation (ESI) interface\textsuperscript{39}. Although these techniques will not be able to evaluate the presence of illicit drug targets in WW quantitatively, it is a significant advance.
for WBDE, specifically for the screening of new psychoactive substances that are continually
developed and have an extensive contribution to the illegal drug market. Furthermore, the
study conducted by Bade et al., demonstrated the value of combining qualitative analysis
through high-resolution MS and SWATH with subsequent quantitative analyses in WBDE\textsuperscript{39}.

\subsection*{9.1.2 FLOW RATE}

Another critical factor is the flow rate of WW through the treatment plant. This factor is
variable day-to-day and can be affected by the precipitation in the area. Heavy precipitation
would result in a dilution of the presence of DTRs in the collected sample. Therefore, it is
essential to obtain flow rate from the treatment plant respective for the 24-hour composite
sample. As this is an important factor, the majority of the studies conducted in the area of
WBDE have taken note that their study has been conducted during the dry season with no
precipitation in order to remove the uncertainty that comes with the added variation of
precipitation\textsuperscript{32,89}. Flow rate data are often obtained from the WWTP where the study is
conducted rather than independently measured. This is because it would reduce costs since
this data are something that the treatment plant readily and consistently measured as part of
protocol. To date, there has been limited study focused on refining flow rate estimates. An
accepted method of obtaining flow rate from WWTPs is the application of a specially tailored
questionnaire that inquires on the standard WWTP protocols on flow rate measurements,
and the active cooperation between WWTP personnel and WBDE investigators.

\subsection*{9.1.3 EXCRETION RATE}

The excretion rate of the selected drug analyte is the proportion of the parent drug that is
converted to the DTR. This is determined by the pharmacokinetics and pharmacodynamics of
the drug\textsuperscript{55,56,60}. The large proportion of the available information on drug analyte excretion data come from studies conducted in the 1980s, or they tend to have a minimal number of subjects\textsuperscript{29}. Based on this, the knowledge of drug-specific pharmacology is quite limited and requires to be updated in order to be effectively utilised in WBDE. Each study in WBDE has also only based their drug excretion data on one set of results, and this is where a meta-analysis of the excretion rate and other pharmacokinetic data from many studies would be useful and provide a more accurate population-derived understanding of these parameters. It has also been discussed that the individuals’ genetics also play a part in the pharmacology of the drug and can be studied through pharmacoepidemiology. However, to conduct studies in drug pharmacology is costly and quite difficult to measure and assess all the parameters involved in the drug’s pharmacology. Additionally, there are ethical considerations when it comes to the study of illicit drug pharmacology on subjects. The added hurdle occurs when it comes to studying pharmacoepidemiology, which would require many subjects to demonstrate and represent the population drug pharmacology adequately. Studies conducted for WBDE utilises excretion rate data based on one drug pharmacology study. Therefore, there is a need for a comprehensive meta-analysis on the pharmacokinetics of the drug, specifically the excretory profiles of illicit drugs and their metabolites that may be DTRs\textsuperscript{26,32,99}. These studies would provide data on the excretion values for various illicit drugs as a mean value but additionally indicate the variation and uncertainty that will occur within a population as per pharmacoepidemiologic studies. Furthermore, as Baker et al. have discussed, evaluate the variations that affect excretion rate due to the various routes of administration\textsuperscript{29}. 
The excretion rate of the drug analyte is a crucial factor for the back-calculation in WBDE, where slight differences in the data would result in magnitudes of error. A considerable emphasis should be put on studies conducted in this area in order to improve the accuracy of WBDE in estimating societal illicit drug use.  

9.1.4 POPULATION ESTIMATION

The premise of WBDE relies on the accurate estimation of the number of people the targeted treatment plant serves. The first studies conducted in WBDE utilised census surveys to obtain the number of individuals in the selected community. There are various flaws with the acquired data as this would also take into account infants and young children who evidently would not be exposed to the use of illicit drugs. Census data also only takes into account those living in homes and not those that are homeless. This is significant, as a recent document published by the Australian Institute for Health and Welfare, assessing the impact of alcohol, and illicit drug use on the burden of disease, have found that those in lower-income situations are more likely to participate in the act of illicit drug abuse. For this reason, estimations on societal illicit drug use have always been underestimated. Due to the high variability in a population that can occur day-to-day; as a result of commuting, holiday periods, et cetera; population estimates cannot be established merely by static census survey results. Therefore, there has been a need to identify another method to estimate the number of contributing persons within the WW catchment, but also assess the fluctuations in population. This concept follows the understanding that there are chemical analytes that are excreted by the human body that may be quantified alongside drug analytes in WBDE as population biomarkers to estimate population numbers. A number of population biomarkers have been proposed. Firstly, water quality parameters, biological oxygen demand, chemical
oxygen demand, phosphorus and nitrogen that are routinely measured by treatment plant personnel, has been utilised the most in studies to indicate population\textsuperscript{22,23,29,45}. However, they have not been validated in their ability to estimate population size. The source of these compounds are not human-specific and can be contributed to WW by industrial means, thus resulting in very large population estimates\textsuperscript{22}. A number of endogenous substances such as creatinine, cortisol, cholesterol, cotinine and 5-hydroxyindoleacetic acid (5-HIAA), were proposed as potential population biomarkers. These, among others, were assessed in a study conducted by Chen et al. as potential population biomarkers by evaluating their stability and prevalence consistency in WW, quantification methods, as well as the correlation between excretion and census population\textsuperscript{101}. In their studies, only cotinine and 5-HIAA were identified as suitable population biomarkers, however cotinine is a metabolite resulting from the metabolism of tobacco\textsuperscript{101}. Therefore, this biomarker is vulnerable to variations based on the culture of smoking habits within the community. Whereas, 5-HIAA is a metabolite of serotonin, an endogenous substance found in all humans, making it a more suitable population biomarker which can also be utilised for universal comparisons. Recently, ammonium has also been proposed as a possible population biomarker\textsuperscript{102}, as it is an indirect marker of urine. Population biomarkers have the potential to provide an indication to the number of individuals within the WW catchment, can be analysed alongside the DTRs, and like WBDE, evaluate the fluctuations in a population that can occur day-to-day, adding to the accuracy of drug use estimates. The limitation that remains still is that population estimates continue to include young children, increasing the uncertainty of drug use estimates. However, this level of uncertainty may always be present in WBDE as there is no method to distinguish between children and adults based on excreted endogenous substances, apart from census data which takes extensive time to collect and assess.
9.2 VALIDITY OF METHODOLOGY

In terms of validation of the methods involved in WBDE, there have not been many studies dedicated towards this area. Most studies do, however, conduct in-study validation of their methods to ensure the quality of data collected.

These studies do so by incorporating isotopically labelled internal standards, specifically deuterated internal standards, to serve as quality controls and reference standards to produce calibration curves. Briefly, a known volume of known incremental concentrations of the reference standard and a fixed volume and concentration of an internal standard is added to make up standard solutions. These standard solutions are analysed, and the ratio of the area generated by the peak of the reference standard to the internal standard is calculated. These points will then form a calibration curve from which unknown concentrations of drug target analytes can be derived. This is done by calculating the ratio between the area under the curve of the peak generated by the analyte to the internal standard. The incorporation of an internal standard is the most beneficial in methods where the volumetric loss of the sample is probable, in order to compensate for uncontrollable changes that occur within the instrument utilised for the analysis. In addition, many studies have also calculated the instrumental detection limits (IDL), instrumental quantification limits (IQL), limit of detection (LOD) and limit of quantification (LOQ) to ensure the that the data collected and reported are within the limits of reasonable reliability.

The stability of metabolites that are the target drug residues utilised in WBDE is an important aspect that continues to require assessment. Several studies conduct metabolite stability assessments alongside their WBDE experiments to demonstrate that the subsequent WW
drug concentrations obtained are reliable by eliminating the variability of DTR degradation\textsuperscript{26,32,89}. The stability of drug metabolites in WW exhibits variability depending on the contents and presentation of certain substances in its environment, and for this reason, concurrent metabolite stability studies prove to be advantageous for that single study. However, this does add a layer of analyses to the experiment that already exhibits many variables. By conducting drug metabolite stability in WW studies\textsuperscript{87,88}, and renewing that knowledge continuously, the study may provide data that can be utilised by many WBDE studies and allow for a standard protocol to be put in place. This additionally may allow for the ease of comparison between communities, cities, states and countries.

10. CONCLUSION

WBDE is an emerging area to study illicit drug use in society. It has demonstrated the potential to objectively evaluate and estimate societal drug use, providing near real-time data and identifying drug use trends within a time course, as well as identifying hot spots of drug use. Utilising WBDE to estimate societal drug use also has the benefit of providing this information non-invasively, which avoids implicating any individual in illegal activity. It has also shown potential in detecting emerging illicit drugs, such as NPSs, by incorporating a secondary method utilising high-resolution MS as a screening method in conjunction with SWATH, data-independent acquisition approach. The information garnered from these studies can assist police to focus their efforts to tackle drug abuse hotspots identified, assist policy makers to develop strategies to deter society in participating in illicit drug use. However, WBDE remains a relatively new area of study and requires further study to refine its methods to obtain further accurate estimates of societal drug use. This literature review has identified some
limitations and areas of WBDE that require more studies dedicated to combat these limitations.

There are four factors that influence the calculation of societal drug use. These include drug concentration in WW, flow rate of WW through the treatment plant, the excretion factor of the DTR and the number of people contributing to the WW catchment. Each of these factors plays an important role in understanding and calculating societal drug use, and therefore it is crucial to obtain accurate data for each component. The limitations regarding in obtaining the drug concentrations in WW lie with the processes pertaining to the sampling, storage, extraction and chemical analyses of the samples.

Studies often report that the results obtained from WBDE are underestimations of actual societal drug use. Poor sorption of drug target analytes during SPE methods will limit what quantitative analyses are able to detect and quantify. A validation study conducted by van Nujis et al. assessed two sorption polymers, Oasis HLB and Oasis MCX, which found that extraction efficiencies and recoveries were generally reproducible for both sorbents, but also found that its effectiveness relied on the chemical profile of the drug target analytes. Further analysis determined that Oasis HLB would be the more favourable given its suitability to acidic, basic and neutral analytes.

Underestimation of drug concentration in WW can also be attributed to the exclusion of DTRs sorption into SPM which are often filtered out and discarded as a step carried out for SPE. A study undertaken by Baker et al. assessed and validated the significance of DTR sorption in SPM by carrying out PLE on WW samples and subsequently analysed using LC-
MS/MS\textsuperscript{94}. The underestimation of drug concentration in water can be due to the unknown processes that occur to the DTR whilst in WW, such as interaction with bacteria and other substances present, and therefore some studies should be directed towards furthering the understanding of the processes that DTR may undergo whilst in WW.

Flow rate data is often obtained through communication and cooperation between WBDE investigators and the WWTP personnel, as this is data readily collected as part of protocol at the treatment plant. At this point, this has been the best practice protocol adopted by investigators\textsuperscript{41,47}. In order to minimise error that comes from the massive influx of influent water that can come from precipitation, most studies are conducted in the dry season and is noted as such or otherwise\textsuperscript{32,45,89,100,104}.

The information on excretion factor of DTRs, mostly metabolites of drugs, has been obtained from studies with limited scope, some conducted in the 1980s and with a limited number of subjects\textsuperscript{29}. For this reason, excretion data should be updated and a meta-analysis should be conducted on the available data, rather than basing WBDE on solitary pharmacokinetic studies. These studies should also be undertaken with a pharmacoepidemiology aspect, additionally assessing the differences in excretion rate as a function of the route of administration\textsuperscript{29,32,99}. However, the likelihood of being able to conduct longitudinal epidemiology studies on the pharmacokinetics of an enormous range of drugs, as well as drugs that are continuously being discovered, is highly unlikely due to the considerable costs and ethical concerns to conduct such studies\textsuperscript{105}. 
The final major parameter contributing to WW analysis is the estimation of population that the WWTP serves. Initially this data was obtained from the design capacity of the WWTP or from census surveys\textsuperscript{25-27}. However this was demonstrated to be inaccurate to utilise static figure as fluctuations in population can occur day-to-day. Studies have been conducted towards identifying possible population biomarkers to estimate population data for WBDE\textsuperscript{45,93,102,106,107}. These studies identifying and evaluating population biomarkers, specifically in relation to WBDE, are all relatively recent. Possible biomarkers that have been proposed include human-specific compounds\textsuperscript{106}, ammonium\textsuperscript{102}, pharmaceuticals and personal care products\textsuperscript{45,93} and genetic biomarkers\textsuperscript{107}. Each of these proposed biomarkers has demonstrated an ability to provide information regarding the number of people served by the catchment. However, a common limitation identified throughout that is significant to WBDE, is the inability to separate the population based on potential drug users based on age groups\textsuperscript{24} and remove individuals that would not be expected to partake in drug use, eg. children.

It is important to realise that for some of these parameters, ideal conditions cannot be met and therefore the only thing that can be achieved is to continuously refine the methods of WBDE in order to objectively and accurately estimate societal drug use. Another limitation of WBDE is the inability to differentiate between individuals based on the level of use, whether they are dependent users or recreational; or whether they are mono- or poly-drug users. Each individual drug use also displays variation in their dosage preference, and so WBDE cannot determine whether estimated drug use is the result of a significant portion of the community engage in casual drug use or whether a small group of illicit drug users are chronic users. Additionally, because drug doses can vary highly between individual, it is difficult to express
results in relation to dose per individual, for example, “dose/day/1,000 persons”. Estimated drug use obtained by WBDE is not stand-alone and can be utilised complementary with other studies, for example, incorporating the findings to a framework to assess the performance of law enforcement\textsuperscript{80}, and therefore as an extension, the performance of policy-makers and the strategies proposed to deter illicit drug use. WBDE has also demonstrated the potential to detect traces of doping by athletes, which will contribute to the sporting field\textsuperscript{50}.

This literature review on the current knowledge of WDBE to estimate societal drug use has demonstrated the capacity and potential of these studies to objectively and directly assess DTRs in WW. This field of study remains in its infancy and continuously requires the addition of new and current knowledge of drug metabolites and its stability in WW; as well as further refinement in the methodology of sample extraction and analysis, flow rate determination, pharmacoepidemiology studies on the pharmacokinetics of drugs and the identification of an appropriate population biomarker.
11. REFERENCES


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PART TWO

Manuscript

Wastewater-based Drug Epidemiology to Estimate Societal Drug Use:
A Critical Review
1. ABSTRACT

Illicit drug use has many consequences resulting in social, health and economic harm. In order to adequately inform the community and law enforcement to efficiently direct their efforts, an objective method of quantifying societal drug use would be useful. To this end, efforts to ascertain societal drug use have relied upon community surveys and extrapolation from law enforcement seizures, which often present the data as biased or skewed due to a small sample size and other associated limitations.

In recent times, wastewater-based drug epidemiology (WBDE) has been proposed as a suitable means to objectively quantify societal drug use. It is a method contingent upon the concept of measuring drug metabolites or biomarkers in wastewater (WW), from which levels of societal drug use are estimated and quantified through extrapolation. The typical methods involve the collection of influent WW from treatment plants where samples are aliquoted and extracted using solid phase extraction (SPE) techniques. The extracted samples are then quantified to obtain drug concentration using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Together with drug concentration, flow rate, drug excretion rate and WW catchment population, an estimate of societal drug use can be obtained through back-calculations. Each of these parameters hold a significant weight in WBDE and require accurate methods in obtaining each parameter.

The aim of this study was to therefore, critically review the various methods of WBDE that have been applied in Australia and in Europe. The outcomes of the assessment pertaining to their validity in directly and objectively measuring societal drug use will be presented.

Keywords: wastewater-based drug epidemiology, wastewater-based epidemiology, illicit drugs, drug abuse, estimate, societal drug use, population
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2. INTRODUCTION

Illicit drug use has become a worldwide epidemic resulting in consequences that have a burden on every facet that makes up a community. It places a burden on the social, medical, policing and economic branches of society. In the period 2004/05, the tangible costs to the economy totalled to just over 6.9 billion dollars, as a result of a reduction in the workforce, absenteeism, premature deaths and illnesses, use of pharmaceuticals, to name a few\(^1\). Of course, not all burdens can be expressed as a monetary loss, illicit drug use can result in the years of life lost (YLL) and the years lived with disability (YLD)\(^{1-3}\). The Australian Institute of Health and Welfare reported that in 2011, illicit drug use resulted in 70,419 years of life lost, equivalent to 3.1\% of all fatal burdens and 31,447 years lived with disability, equivalent to 1.4\% of all non-fatal burdens\(^3\). Illicit drug use has been shown to affect the individual’s interaction with society, resulting in a detrimental disconnection to the world around them. Although illicit drug use has been shown to be stable across the recent years with the National Drug Strategy Household Survey of 2016 reporting a similar proportion of lifetime use to that reported in 2013, 43\% and 42\% respectively. However, the NDSHS also note that illicit drug use has gradually increased since 2001, at which point 38\% of the Australian population aged 14 or older had used illicit drugs at some point of their life\(^4\). Between 1998/99 and 2004/05, there has been an 11.3\% increase in total cost of illicit drug use, when assessed using 2004/5 prices\(^1\). It is important to understand that the culture of illicit drug use has drastically changed across the space of decades, with the decline in the prevalence of some illicit drugs and the emergence of others, in particular, novel psychoactive substances (NPS). NPSs are synthetic chemicals that are manufactured to mimic the effects of existing psychoactive drugs, such as cannabinoids and ecstasy. The manufacture of NPSs occurs
rapidly and is easily achieved as it involves a minor adjustment to the chemical structure, often making it undetectable to many screening methods. It is for this reason that most NPSs avoid legislation.

Understanding the true societal drug use is highly important. Accurately assessing real-time societal drug use will allow for the effective direction of the efforts of the various key community groups; law enforcement, medical professionals and government bodies such as policy makers. The negative ripple effect that illicit drug use produces starts with the individual and extending out to friends and family and then to the rest of the community. This has led to the prohibition of many drugs. By accurately estimating the level, relative proportion and prevalence of various illicit drug use, it could help bring a greater awareness to the drug epidemic. This understanding of societal drug use can be used in conjunction with policy frameworks to assess the success of these policies that have been put in place to combat the drug use epidemic. Additionally, understanding the current trends and prevalence of various drugs, can assist medical and rehabilitation services to effectively budget and regulate programs.

Past methods of measuring societal drug use was dependent on community and government surveys\textsuperscript{4,6-10}. Self-reporting surveys are often open to bias as they are reliant on the individual’s recollection of events pertaining to drug use. This problem is compounded if the individual is under the influence whilst taking part in the survey, as the physiological and psychological effects of drugs can impact an individual’s recollection\textsuperscript{11,12}. Another factor that places additional uncertainty in surveys is that often individuals are reluctant to be completely honest with their participation in illicit drug use, in fear of being reprimanded for their actions,
since such actions are illegal\textsuperscript{13}. Surveys have a recurring problem of returning unresponsive, with only 1\% of a population of 29,083, completing the survey in a study conducted assessing the participation in voluntary surveys\textsuperscript{14}. Additionally, conducting surveys, distributing and analysing them thereafter has been demonstrated to be costly\textsuperscript{15}. Therefore, police and medical reports are also collected to measure societal drug use, but these also present a bias; police reports will evidently report on the illegal activities, whereas medical reports are often restricted due to doctor-patient confidentiality. In order to accurately assess the level of societal drug use, such data should be collected utilising methods that are able to present such data in an unbiased and objective manner. To this extent, WBDE has been proposed as a means to do so.

2.1 WASTEWATER-BASED DRUG EPIDEMIOLOGY

Wastewater-based epidemiology (WBE) is the study pertaining to the analysis of wastewater (WW) to understand the incidence and distribution of factors relating to the health and welfare of a community. Furthermore, wastewater-based drug epidemiology (WBDE) is the study pertaining to the analysis of WW to understand the incidence and distribution of drug use. The concept of WBDE is impinging on the understanding that consumed drugs are metabolised and eliminated from the body, and these eliminated factors are drug biomarkers or metabolites that are present in urine and therefore in WW. It is then possible to quantify this and obtain drug concentration in WW. Together with other important parameters; flow rate, drug excretion rate and WW catchment population; through back-calculation, an estimate on societal drug use can be obtained. It has the potential to provide close to real-time estimate on societal drug use, assess trends and identify drug use hotspots. WBDE studies are non-invasive and protects the privacy of all individuals, collecting information
without incriminating any one individual, thus providing an unbiased understanding of societal drug use. The concept was first proposed by Daughton et al. with the aim to assess the effect of drug excretion products to bring awareness of societal illicit drug use to the community and its impact on the environment, for example, aquatic life\textsuperscript{13}. Since then, studies have utilised WBDE as a means to assess and quantify the level of societal drug use. Zucatto et al. laid out the first initial studies contributing to WBDE and assessed cocaine use in Italy by back-calculating using four factors: drug concentration quantified in WW, flow rate of WW through the treatment plant, excretion rate of the drug target residues (DTR) and the number of people served by the WWTP catchment\textsuperscript{16}. Since then, many studies have been conducted with the aim of optimising and refining these robust methods laid out by Zucatto et al. towards a method that provide greater accuracy on the understanding of societal drug use. WBDE studies are still in their infancy, with most of the studies having been conducted within the 15 years, but WBDE demonstrates a great potential to contribute to the knowledge of societal drug use.

3. DISCUSSION

WBDE is the study and analysis of WW to understand the incidence and distribution of drug use that affects societal health and welfare. The concept of WBDE is impingent on the understanding that the human consumption of illicit drugs undergoes metabolism within the body and is eliminated\textsuperscript{13}. Biomarkers and metabolites of the illicit drug are eliminated from the human body and are present in urine and therefore expected to be present in WW\textsuperscript{13}. It is these biomarkers and drug metabolites that are the DTRs for WBDE analysis. In WBDE, there are four parameters that are paramount to the back-calculations used to estimate societal drug use. These are, drug concentration derived from the quantitation of DTR (5.1), flow rate
of WW through the (WWTP) (5.2), drug excretion rates from the understanding of drug pharmacology (5.3) and population served by WWTP catchment (5.4). Each of these parameters play an important and crucial role in WBDE and therefore require optimised methods to accurately obtain the relevant data. These parameters have factors that influence the accuracy of the data obtained for them. This review aims to assess the methods involved in obtaining each parameter.

3.1 DRUG CONCENTRATION

Drug concentration is derived from the quantitation of DTRs within WW. Factors that affect the accuracy of drug concentration are the sampling, preparation and storage protocols (5.1.1), DTR selection (5.1.2) and sample extraction and chemical analysis (5.1.3). Each of these plays a crucial role in obtaining drug concentration.

3.1.1 SAMPLE COLLECTION, PREPARATION AND STORAGE

This is the very first step in WBDE and sets the foundation for the remainder of the study. In order to obtain a representative sample of the day, a 24-hour composite sample is obtained of influent water utilising a flow- or volume-proportional automatic sampling device with short sampling intervals. The decision to choose influent WW samples over effluent WW samples is often in relation to bypassing the risk of DTRs being filtered or removed by the WWTP before it can be quantified. Collecting influent WW samples can be useful in cases assessing the effectiveness of WWTP in removing illicit drug residues from WW or assessing the effects of remnant illicit drug residues on the environment. A study conducted by Castiglioni et al. assessed the uncertainties involved with WBDE and with respect to sampling devices, they had concluded that in order to avoid bias and minimise the relative standard
deviation involved with sample collection, a flow- or volume-proportional automatic sampling device with short intervals would be the most effective. An alternative sampling device had been proposed, a polar organic chemical integrative sampler (POCIS) which was demonstrated to recover some illicit drug analytes present in effluent water\textsuperscript{18} but also exhibited the limitation in that only polar drug analytes are detected by this device. This would result in the misrepresentation of the results to estimate societal drug use. This device may be useful if a specific drug is the subject of a WBDE and has a polar DTR that can be detected by POCIS. Once 24-hour composite samples have been collected, analytical subsamples are derived from the original composite sample. These analytical subsamples are acidified to reduce the risk of in-sample transformations\textsuperscript{19} and kept refrigerated in the dark at 4 °C if the sample will be analysed within 3 days\textsuperscript{16,20,21}, or at -20 °C if samples are analysed within 2 months of collection\textsuperscript{16,22-24}.

3.1.2 SELECTION OF DRUG TARGET ANALYTES

WBDE is based on the understanding that drugs that are consumed will undergo metabolism within the human body and eliminated as biomarkers of the drug or its metabolites, with a small fraction possibly remaining as the parent drug. Each of these possible drug analytes have different pharmacological profiles and therefore behave differently. It is important to select an appropriate one in order to accurately estimate societal drug use. Several criteria must be met in order to be selected as an appropriate DTR for the illicit drug in question. The DTR must be stable in WW\textsuperscript{19,23,25-28}, must be a significant excretion product\textsuperscript{25}, have sufficient pharmacological excretion data\textsuperscript{25,29} and have a chemical profile that has a reasonable recovery rate\textsuperscript{30}. A study conducted by Castiglioni et al. assessed cocaine and its metabolites as potential DTR for WBDE and found that major cocaine metabolite, benzoylcegonine (BZE)
was the best suited DTR for WBDE based on its occurrence and stability in WW\textsuperscript{30}. This study also noted differences in stability assessments of DTR between studies\textsuperscript{23,30} where the experimental conditions were the same, and the only difference was the WW samples. This suggests that the nature and composition of WW highly influences the results that can be obtained from samples. To that end, DTR stability should be assessed within each study as a means to ensure the results that will be obtained from the study are reliable.

3.1.3 SAMPLE EXTRACTION AND CHEMICAL ANALYSIS

The most common extraction and subsequent quantitation methods that has become the mainstay of WBDE is SPE techniques and quantitation by means LC-MS/MS\textsuperscript{16,23,25,29,31-35}. Most of these studies utilised high-performance liquid chromatography (HPLC) coupled to a triple quadruple (QqQ) mass spectrometer equipped with electrospray (ESI)\textsuperscript{16,35-37}. Variations in the LC-MS/MS apparatus used; for example, high-performance or ultrahigh-performance LC, or triple quadruple or QTrap mass spectrometer, etc; has not been thoroughly evaluated and at this stage is not thought to result in a significant variation but is something that should be looked at in future studies.

Some studies have identified limitations of WBDE that require additional methods to obtain a representation and estimate of whole societal drug use. A limitation that has been identified is the omission of suspended particulate matter (SPM) and the possibility of the sorption of DTR in SPM\textsuperscript{17,38,39}, as it is often filtered from the samples before extraction techniques are performed\textsuperscript{20}. This lead to the incorporation of pressurised liquid extraction (PLE) techniques to overcome this hurdle\textsuperscript{38}. There is also conflicting evidence that the omission of the sorption of DTR in particulates for calculations does not impact the estimate enough to justify an extra
method to isolate SPM\textsuperscript{13,32}. A continuous limitation that has been identified with the utilisation of LC-MS/MS techniques is that this method only allows for the quantitation of drug analytes that are actively under inquiry. Those that are not actively screened for are often missed. To overcome this, a qualitative method utilising high-resolution MS has been proposed to be incorporated into WDBE studies\textsuperscript{36,40-42}. A recent study conducted in Australia employed both a qualitative and quantitative method, but also evaluated the use of a data-independent acquisition method, Sequential Window Acquisition of all THeoretical fragment-ion spectra (SWATH)\textsuperscript{35}. SWATH is a data acquisition method that has a much narrower window of fragmentation, and therefore is able to separate fragment ions with greater discriminatory power. Utilised in conjunction with high-resolution MS, it allows for the qualitative screening and detection of all possible DTRs that are present in the sample. This method has primarily been used in the study of proteomics but has shown promising results in its utilisation for societal drug use estimate in WBDE, specifically in its ability to screen for NPS\textsuperscript{35}.

### 3.2 FLOW RATE

The rate at which WW flows through the WWTP is an important factor that is required for the back-calculation in WBDE. This factor can vary day-to-day and is influenced by environmental factors, specifically precipitation, which is why majority of WBDE studies aim to be conducted during the dry weather season where the chance of precipitation to circumvent this added variability\textsuperscript{17,23}. Flow rate data is often obtained from the WWTP as flow rate is routinely measured as part of the protocol. This would reduce costs for the study. It is therefore important to accurately obtain flow rate data from the WWTP and the accepted method to do so is through a specially tailored questionnaire, inquiring of the standard flow rate
measurement protocols of the WWTP, and the active and ongoing cooperation between the WWTP personnel and investigators\textsuperscript{43}.

### 3.3 Excretion Rate

The underlying concept of WBDE is the understanding that drugs consumed by the human body are metabolised and broken down to their metabolites\textsuperscript{13}. These metabolites are eventually eliminated from the body through urine. These metabolites and biomarkers are drug analytes targeted for WBDE studies\textsuperscript{13}. The excretion rate of these selected DTRs describes the proportion of the parent drug that is converted to its metabolites or biomarkers. Excretion rate is a critical factor for WBDE, used in the back-calculation from the drug concentration detected in WW to an estimated total drug use load by the community. Excretion rate is a factor that is determined by the pharmacokinetics and pharmacodynamics of the drug\textsuperscript{44-46}. The data derived from these studies exhibit many limitations such as being outdated, most having been conducted in the 1980s, or that they have minimal subjects\textsuperscript{29}. WBDE studies have only based their pharmacologic excretion rates on a singular study, where rather, a meta-analysis of drug excretion rates would be more appropriate\textsuperscript{17,25,47}. The knowledge of illicit drug-specific pharmacology is quite limited and requires not only an update and a larger sample size, but also an extension, specific to illicit drug use, is the exhibited variations in excretion rate as a result of various routes of administration\textsuperscript{29}. A large sample size is important as a population may exhibit different drug pharmacological profiles to another. This concept is pharmacoepidemiology, which is the study of the incidence and distribution of pharmacological factors within a population. Advancing and conducting any pharmacological study requires many ethical considerations and are often difficult to set a control. Conducting these large-scale studies in order to hopefully accurately represent a
population requires a lot of monetary and time investment; however, it is emphasised that drug excretion rate, and drug pharmacology are extremely important factors to obtain accurate data in order to improve the accuracy of WBDE.\textsuperscript{29}

\textbf{3.4 POPULATION ESTIMATE}

The premise of WBDE relies on the accurate estimation of the population that the WWTP serves. Initially, studies conducted in WBDE utilised census surveys to obtain the number of people within the selected community, or the estimated number of individuals within catchment from the WWTP. Various flaws follow this assumption, one being that the population estimation derived from both methods take into account infants and young children who would evidently be exempt from the use of illicit drugs. Secondly, census data would only take into account those living in homes and not those that are homeless. This is significant, as the socio-economic group most likely to partake in the actions of illicit drug use are those who are of a lower-income status.\textsuperscript{3} And finally, these population estimates are static, but population numbers fluctuate day-to-day as a result of commuting, travelling due to holiday periods, et cetera.\textsuperscript{48-50} Therefore, there have been studies aiming to identify a suitable population biomarker in WW to accurately estimate population. The concept is similar to that underpinning WBDE, an understanding that there is a chemical analyte excreted by the human body that may allow an accurate population estimate. Several population biomarkers have been proposed. Most common is the use of water quality parameters such as biological oxygen demand, chemical oxygen demand, phosphorus and nitrogen, routinely assessed by WWTP personnel;\textsuperscript{29,33,50,51} however, these have not been validated in their ability to accurately estimate population size. Additionally, the source of these chemical analytes are not solely human, and can be derived from industrial processes.\textsuperscript{33}
Endogenous substances\(^4\), ammonium\(^5\), an indirect biomarker for urine, and genetic biomarkers\(^6\) have been suggested as alternative population biomarkers. Each of these show promising results but have yet to be validated as accurate methods to estimate fluctuating population both short-term and long-term. Obtaining an accurate population is a difficult hurdle for WBDE, specifically to estimate societal illicit drug use in that population estimates through population biomarkers cannot distinguish from young children. Population fluctuations occur randomly and to varying degrees, and so may inherently present constant level of uncertainty.

### 3.5 Validation of Methods

WBDE studies are still very much in their infancy, and so there have not been a large number dedicated solely to the validity of these studies in their accuracy to estimate societal drug use\(^2\). However, many studies conduct in-study validation of their methods to ensure the quality of the data that has been collected and analysed\(^3\). These studies incorporate isotopically labelled internal standards, specifically deuterated internal standards, to serve as quality controls and reference standards to produce calibration curves\(^4\). From this, the unknown concentrations of the DTRs can be derived. The incorporation of internal standards is quite suited WBDE, specifically as its methods involve techniques where volumetric loss of the sample is probable. It allows for the compensation of the uncontrollable changes that occur within the instrument utilised for the analysis. In addition, studies would also incorporate calculations to portray the uncertainties related to the instrument. These studies calculate the parameters, instrumental detection limits (IDL), instrumental quantification limits (IQL), limit of detection (LOD) and limit of quantification (LOQ) to ensure that the data obtained from the analysis are within the realms of reasonable
reliability\textsuperscript{16,22,23,25}. There is a need for validation studies for WBDE as this area of study continues to evolve and requires method validity to ensure the reliability of these studies to objectively derive estimates of societal drug use.

4. CONCLUSION

WBDE is an emerging area of study that can assess the extent of drug use in society. It has demonstrated the ability to objectively evaluate and estimate societal drug use, providing close to real-time data, identifying trends and hotspots of drug use. WBDE is a non-invasive method and has the benefit of providing information on drug use without implicating any one individual in the illegal activity\textsuperscript{13}, additionally demonstrated the potential to detect traces of doping by athletes in the sporting field\textsuperscript{57}. Policy makers and law enforcement also have the ability to incorporate WBDE and its findings into a framework to assess the success of strategies that have been put in place to combat illicit drug use in the community\textsuperscript{5}. It has also shown the potential to detect and analyse drug analytes derived from NPSs\textsuperscript{35}, a newer niche of illicit drugs available that often avoid legislation due to the minor structural changes of existing psychoactive substances. This is done by incorporating a qualitative method, utilising a high-resolution mass spectrometer equipped with SWATH, a data-independent acquisition method commonly used in proteomics, to screen for compounds that are not always initially targeted\textsuperscript{35}. The information that is obtained from WBDE has the ability to assist police, health and medical facilities and government groups to effectively direct their efforts.

So far, studies in WBDE have underestimated the level of societal drug use, indicating that either past methods of social drug use estimates through census surveys, police and medical records, have been overestimated, and/or methods of WBDE require refinement in order to
obtain reliable, objective and accurate estimates of societal drug use. Many studies have identified limitations to WBDE studies and areas of improvement required for the measurement of each parameter. A factor that has been identified contributing to underestimation of societal drug use, is the omission of DTR sorption into SPM, solid matter often filtered from the sample before extraction occurs\textsuperscript{20}. To combat this, PLE techniques have been incorporated with the aim to encompass all forms of DTR found in WW\textsuperscript{29,30,58}. Another level of uncertainty possibly contributing to the underestimation of social drug use, is the estimation of population served by the WWTP. The usage of static population numbers provided by census surveys or by WWTP catchment capacity, have shown to produce underestimates when utilised in back-calculations for WBDE\textsuperscript{16,25,59}. For this case, population biomarkers that can be found in WW has been proposed as a method to measure population\textsuperscript{48,50,52,53}. This approach has demonstrated potential when incorporated into WBDE, especially in short-term studies\textsuperscript{52}. This is particularly advantageous over static population estimates as population is known to fluctuate, even day-to-day as a result of commuting, holiday periods, et cetera. Studies in population biomarkers are novel, requiring more study contributions and validation in its accuracy to accurately estimate population.

The pharmacology aspect of WBDE is an area that exhibits a deficit in knowledge, requiring an update and refinement of excretion factors that are utilised for the back-calculation of societal drug use. It has been suggested that a large-scale pharmacoepidemiology study and meta-analysis on illicit drugs is required, with a key to acknowledge the differences in excretion rate relative to the various routes of administration\textsuperscript{17,29,47}. However, many studies have acknowledged that the ability to conduct
these longitudinal drug pharmacoepidemiology studies would be strenuous, consuming a large amount of money and resources\textsuperscript{60}.

It is important to acknowledge that some of these parameters, ideal conditions cannot be met and therefore WBDE can only move forward with continuous refinement of its methods to obtain as accurate and objective as possible estimates of societal drug use. A drawback of WBDE is that it cannot distinguish between recreational drug use and chronic and dependent drug use, or whether the individual uses various drugs or has a preferential use of one drug. Each individual drug user have their own preferences and their own dosage, making it difficult to estimate societal drug use based on drug dose.

WBDE to estimate societal drug use has demonstrated a great potential to contribute to the understanding of illicit drug use within a community. The studies that have contributed to the current knowledge of WBDE have been conducted within the last decade and a half, establishing itself as an area still in its infancy. Future studies would require the advancement and refinement in each facet of WBDE, with a focus on drug pharmacoepidemiology studies, and studies to refine population estimates.
5. REFERENCES


