Illicit stimulant use in the population monitored by wastewater analyses

Chang Chen (M. Med.)

Discipline of Pharmacology, School of Medical Sciences

The University of Adelaide

August, 2013

A thesis submitted for the degree of Doctor of Philosophy

This thesis is dedicated to my Mom and Dad

谨以此论文献给我的父母

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution in my name and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

PUBLICATIONS INCLUDED IN THIS THESIS

Chen C.*, Kostakis C., Irvine R. J., Felgate P. D., White J. M. (2013). Evaluation of pre-analysis loss of dependent drugs in wastewater: stability and binding assessments. Drug Testing and Analysis, 5(8): 716–721.

Irvine R. J., Kostakis C., Felgate P. D., Jaehne E. J., **Chen C.***, White J. M. (2011). Population drug use in Australia: a wastewater analysis. Forensic Science International, 210(1–3): 69–73.

Chen C.*, Kostakis C., Harpas P., Felgate P. D., Irvine R. J., White J. M. (2011). Marked decline in 3,4-methylenedioxymethamphetamine (MDMA) based on wastewater analysis. Journal of Studies on Alcohol and Drugs, 72(5): 737–740.

Chen C.*, Kostakis C., Irvine R. J., White M. J. (2013). Increases in use of novel synthetic stimulant are not directly linked to decreased use of 3,4-methylenedioxy-N-methylamphetamine (MDMA). Forensic Science International, 231(1–3): 278–283.

*** Corresponding author**

…… Chang Chen, 16/8/2013

TABLE OF CONTENTS

ABSTRACT ..... **i**

ACKNOWLEDGEMENTS ..... **iii**

STATEMENT OF AUTHORSHIP AND CONTRIBUTION ..... **v**

ABBREVIATIONS ..... **xii**

CHAPTER 1 Introduction **1**

1. Historical and contemporary stimulant abuse 4
   1.1. Cocaine 4
   1.2. Methamphetamine 5
   1.3. MDMA 6
   1.4. Novel synthetic stimulants 7
2. Pharmacology of illicit stimulants 10
   2.1. Cocaine 11
   2.2. Methamphetamine 12
   2.3. MDMA 13
   2.4. Novel synthetic stimulants 15
3. Social burden of illicit drug use 16
   3.1. Health-related issues 17
   3.2. Crime 18
   3.3. Social capital costs 19
4. Current monitoring methods 20
   4.1. Significance of drug control policies 20
   4.2. Significance of information on illicit drugs 22
   4.3. Surveys 23
   4.4. Reporting systems 24
   4.5. International reports 27
   4.6. Summary 28
5. Wastewater epidemiology approach 29
   5.1. Origin 29

5.2. Metabolic and pharmacokinetic foundations 30
5.3. Methods 41
5.4. Applications 43
5.5. Advantages and current issues 50
6. Research aims 52
6.1. Method validation: evaluation of pre-analysis loss 53
6.2. Application in South Australia 54
6.3. Monitoring of MDMA use 54
6.4. Novel synthetic stimulants in wastewater 54

CHAPTER 2 "Evaluation of pre-analysis loss of dependent drugs in wastewater: stability and binding assessments" 56

CHAPTER 3 "Population drug use in Australia: a wastewater analysis" 73

CHAPTER 4 "Marked decline in 3,4-methylenedioxymethamphetamine (MDMA) based on wastewater analysis" 80

CHAPTER 5 "Increases in use of novel synthetic stimulant are not directly linked to decreased use of 3,4-methylenedioxy-N-methylamphetamine (MDMA)" 85

CHAPTER 6 Discussion 98

1. Summary of major achievements 98
1.1. Method validation: evaluation of pre-analysis loss 98
1.2. Application in South Australia 100
1.3. Monitoring of MDMA use 101
1.4. Novel synthetic stimulants in wastewater 102
2. Conclusion 103
3. Future research 104

BIBLIOGRAPHY 106

LIST OF FIGURES

Figure 1. Chemical structures of stimulants studied in this thesis 3
Figure 2. Metabolic pathways of cocaine in humans. 33
Figure 3. Metabolic pathways of methamphetamine in humans 34
Figure 4. Metabolic pathways of MDMA in humans 36
Figure 5. Metabolic pathway of methcathinone in humans 37
Figure 6. Proposed metabolic pathways of mephedrone in humans 37
Figure 7. Proposed metabolic pathways of methylone in humans 38
Figure 8. Proposed metabolic pathways of MDPV *in vitro* 38
Figure 9. Proposed metabolic pathways of BZP in humans 39
Figure 10. Proposed metabolic pathway of TFMPP in humans 40
Figure 11. Current procedures of wastewater epidemiology studies 43

LIST OF TABLES

Table 1. Studies published by 2009 on the analyses of illicit drugs and metabolites in wastewater and surface water 44

ABSTRACT

Illicit stimulant use is a global problem, and accurate and timely information on population stimulant use is essential. However, traditional monitoring methods based on surveys and reporting systems have some limitations, which include the accuracy of the data collected, its relevance to the population as a whole, and the long delays in reporting in a rapidly changing drug scene. These limitations are problematic when using the collected data to develop health and policing policies. Analysis of community wastewater has been suggested to be an objective and quick method to provide supplementary data. However, a detailed knowledge of the pharmacology and chemistry of drugs is required combined with development and validation of techniques before this method is universally applied.

The general aims of this thesis were to develop and validate the method of wastewater analysis, and then apply it to monitor illicit stimulant use in the general population. The analytical method used in this study was mainly solid-phase extraction coupled with liquid chromatography- tandem mass spectrometry.

Firstly, pre-analysis loss of drugs and metabolites were evaluated. The results from this study showed that negligible loss of the studied drugs occurred after filtration, and all analytes except cocaine and 6-monoacetylmorphine are relatively stable in wastewater. For cocaine use monitoring, its metabolite benzoylecgonine is a more suitable analytical target for stability reasons. To stabilise 6-monoacetylmorphine, the addition of sodium metabisulphite is recommended.

Secondly, wastewater analysis was applied to monitor the use of illicit stimulants cocaine, methamphetamine and 3,4-methylenedioxy-N-methylamphetamine (MDMA) in the State of South Australia, Australia. Data were collected for international comparisons, geographical comparisons and weekly use pattern assessments.

Continued monitoring of the wastewater revealed a decline in MDMA use in Adelaide, which provided objective data to support the globally reported MDMA shortage starting from 2009.

Since late 2000s, there has been an increase in the use of novel synthetic stimulants. It was suggested that this increase might be associated with the MDMA decline, but it was also possible that these two phenomena were not related. To verify these hypotheses, an analytical method for the analysis of some of the most reported synthetic stimulants was developed, validated and applied. Results showed some sporadic increases in the use of new synthetic stimulants during 2010 and 2011, but these increases were not directly linked with the MDMA decline, suggesting the novel synthetic stimulants have not replaced MDMA.

In conclusion, this thesis developed and validated the method of wastewater analysis and gained important information on stimulant use in the population, which cannot be obtained via other monitoring approaches.

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my deepest thanks to my supervisors, Associate Professor Rodney Irvine, Professor Jason White, Chris Kostakis and Dr Abdallah Salem. Professor White and Associate Professor Irvine provided me not only with this great opportunity to come to Australia and participate in this exciting research project, but also with all the academic, financial and emotional support that I can imagine. Chris passed me all the skills I needed in this project and took care of me just like an elder brother. Thanks are also due to Dr Salem for his help throughout my PhD, particularly after the retirement of Associate Professor Irvine.

This project would not have been finished without the support from many other people. Just to name a few:

- Colleagues at the University of Adelaide: Professor Andrew Somogyi, Professor Paul Rolan, Dr Femke Buisman-Pijlman, Dr Janet Coller, Dr Mark Hutchinson, Dr Scott Smid, Dr Ian Musgrave, Gordon Crabb, Karen Nunes-Vaz, Dr Yue Wu, Dr Emily Jaehne, Dr Irina Majumder, Dr Peter Grace, Dr Liang Liu, Yibai Li, Yuen (Heilie) Kwok, Nicole Sumracki, Benjamin Harvey, Jacob Thomas, James Swift, Jake Gordon, Eloise Gelston, Jacinta Johnson, Muhammad Imran Ahmad and Intan Omar.
- Staff at SA Water and Allwater: Dr Andrew Humpage, Ian Mackenzie, Bronwyn Kent, Naomi Edwards, Stacey Smith, Jim Rozaklis, Daniel Squire, John Jamieson, Gary Lansdale, Phil Wootton, Vince Spadavecchia, Frank Nemeth, Garry Launer, Steve

Kennedy, Ronnie O'Lochlin, Craig Heidenreich, Mike Gallant, Steve Ansell, Darran Clark, Wayne Amme, Tom Richards and Arty Cassions.

- Staff at Forensic Science South Australia: Peter Felgate, Peter Harpas, Peter Stockham, Penny Kostakis, Christine Nash, Marc Grabowski, Kerryn Mason, Danielle Butzbach, Michaela Kenneally, Lauren Geier, Trish Smith, Tom Woods, Tim Scott, Emma Partridge, Heather Felgate, Heather Lindsay, Peter Byass, Tanya Cooper, Alan Pollnitz, William Traljic, Liz Gully, Amanda Thompson, Joanna Rositano, Andrew Camilleri and Justin Granleese.
- Staff and students at University of South Australia: Dr Cobus Gerber, Benjamin Tscharke, Associate Professor Robert Milne, Dr Tomas Rozek, Professor Shudong Wang and Ben Noll.

My parents and relatives in China gave me unending love and support in every aspect that I can never thank enough. I must also thank my girlfriend Xin Xin for her trust, encouragement and patience. Tongzhi Wu, Lifang Zhong, Lijuan Wei, and all the other friends of mine, thank you for your company and support during this unforgettable period of time!

I also appreciate The China Scholarship Council- University of Adelaide Joint Scholarship, Faculty of Health Sciences Research Committee Postgraduate Travelling Fellowships, School of Medical Sciences Postgraduate Travel Awards, ASCEPT Student Travel Awards and The Government of South Australia for financial support.

STATEMENT OF AUTHORSHIP AND CONTRIBUTION

Chen C., Kostakis C., Irvine R. J., Felgate P. D., White J. M. (2013). Evaluation of pre-analysis loss of dependent drugs in wastewater: stability and binding assessments. Drug Testing and Analysis, 5(8): 716–721.

Mr Chen (candidate) designed and performed the experiments, interpreted the data, wrote the manuscript and submitted it for publication.

Signed …… *Date*……

Mr Kostakis was involved in the experimental design and supervision, and evaluated the manuscript.

Signed …… *Date*……

Associate Professor Irvine was involved in the data interpretation and manuscript preparation.

Signed …… *Date*……

Mr Felgate was involved in the sample analysis and manuscript evaluation.

Signed …… *Date*……

Professor White was involved in the data interpretation and contributed to the manuscript preparation.

Signed …… *Date*……

Irvine R. J., Kostakis C., Felgate P. D., Jaehne E. J., **Chen C.**, White J. M. (2011). Population drug use in Australia: a wastewater analysis. Forensic Science International, 210(1–3): 69–73.

Mr Chen (candidate) was involved in the sample and data analysis, graphical presentation of the data and manuscript preparation, and submitted the manuscript for final publication.

Signed …… *Date*……

Associate Professor Irvine was involved in the experimental design and sample collection, and had a major input in the manuscript preparation.

Signed …… *Date*……

Mr Kostakis developed the analytical method and had a major contribution to the sample analysis. He was also involved in the data interpretation and manuscript preparation.

Signed …… *Date*……

Mr Felgate was involved in the sample analysis and manuscript preparation.

Signed …… *Date*……

Dr Jaehne conducted the majority of the statistical analysis and graphical presentations of the collected data.

Signed …… *Date*……

Professor White was involved in the experimental design, sample collection and manuscript preparation.

Signed …… *Date*……

Chen C., Kostakis C., Harpas P., Felgate P. D., Irvine R. J., White J. M. (2011). Marked decline in 3,4-methylenedioxymethamphetamine (MDMA) based on wastewater analysis. Journal of Studies on Alcohol and Drugs, 72(5): 737–740.

Mr Chen (candidate) had a major contribution to the sample and data analysis, as well as manuscript preparation and submission.

Signed …… *Date*……

Mr Kostakis was involved in the sample collection and analysis, and contributed to the manuscript preparation.

Signed …… *Date*……

Mr Harpas provided roadside drug testing data in this manuscript.

Signed …… *Date*……

Mr Felgate was involved in the sample analysis and manuscript preparation.

Signed …… *Date*……

Associate Professor Irvine was involved in the manuscript evaluation.

Signed …… *Date*……

Professor White designed the study and critically evaluated the manuscript.

Signed …… *Date*……

Chen C., Kostakis C., Irvine R. J., White M. J. Increases in use of novel synthetic stimulant are not directly linked to decreased use of 3,4-methylenedioxy-N-methylamphetamine (MDMA). Forensic Science International, 231(1–3): 278–283.

Mr Chen (candidate) had a major contribution to the method development, sample and data analysis, and manuscript preparation and submission.

Signed …… *Date*……

Mr Kostakis was involved in the method development, sample analysis and manuscript preparation.

Signed …… *Date*……

Associate Professor Irvine was involved in the manuscript evaluation.

Signed …… *Date*……

Professor White critically evaluated the manuscript.

Signed …… *Date*……

ABBREVIATIONS

5-HT: 5-hydroxytryptamine (serotonin)

AIDS: acquired immunodeficiency syndrome

AMP: amphetamine

ATS: amphetamine-type stimulant

BE: benzoylecgonine

BZP: benzylpiperazine

CNS: central nervous system

COC: cocaine

COD: codeine

COT: cotinine

CRS: cocaine-related substance

DA: dopamine

EDRS: Ecstasy and Related Drugs Reporting System

EMCDDA: European Monitoring Centre for Drugs and Drug Addiction

GC: gas chromatography

GDP: gross domestic product

HHA: 3,4-dihydroxyamphetamine

HHMA: 3,4-dihydroxymethamphetamine

HILIC: hydrophilic interaction liquid chromatography

HPLC: high performance liquid chromatography

IDRS: Illicit Drug Reporting System

LC: liquid chromatography

LLE: liquid-liquid extraction

ITMS: ion trap mass spectrometer

LVI: large-volume injection

MA: methamphetamine

MAM: 6-monoacetylmorphine

MDA: 3,4-methylenedioxyamphetamine

MDMA: 3,4-methylenedioxy-N-methylamphetamine

MDPV: methylenedioxypyrovalerone

MOR: morphine

MS: mass spectrometry

MTD: methadone

$Na_2S_2O_5$: sodium metabisulphite

NDARC: National Drug and Alcohol Research Centre

NE: norepinephrine

NIDIP: National Illicit Drug Indicators Project

POCIS: polar organic chemical integrative sample/sampler

RSD: relative standard deviation

SPE: solid-phase extraction

TFMPP: 3-trifluoromethylphenylpiperazine

TQMS: triple quadruple mass spectrometer

UNODC: United Nations Office on Drugs and Crime

UPLC: ultra performance liquid chromatography

WHO: World Health Organisation

WWTP: wastewater treatment plant

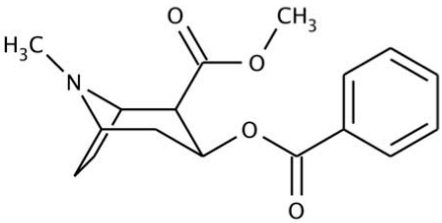
CHAPTER 1 Introduction

Use of illicit drugs is a severe social problem faced by almost all nations in the world. According to the World Drug Report 2012 (UNODC 2012), one in every twenty adults in the world used illicit drugs in 2010, of which 0.6% were problem users. This prevalence results in 0.2 million deaths annually, which are associated with thousands of family tragedies and miseries. Social capital cost directly or indirectly related to the production, trafficking and consumption of illicit drugs is also enormous. Crimes and transmitted diseases such as acquired immunodeficiency syndrome (AIDS) resulting from needle sharing among injecting users are additional consequences of illicit drug use (UNODC 2012).

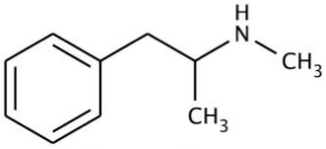
Illicit drugs can be categorised into cannabis, opioids, stimulants, hallucinogens, inhalants, steroids, non-medical use of prescription drugs, etc. Among all these drugs, stimulants are a special group for two reasons. Firstly, their use is frequent in the population, with Australia suffering the globally highest prevalence of amphetamine-type stimulants (ATS), including methamphetamine and 3,4-methylenedioxy-N-methylamphetamine (MDMA) (UNODC 2012). Secondly, the usage pattern of stimulants can change quickly over time (UNODC 2012), making it difficult to obtain timely and accurate information on their prevalence via traditional monitoring methods.

The present chapter aims to explain how this thesis contributes to the challenges mentioned above. Firstly, historic and contemporary situations of illicit stimulant use are introduced, followed by their pharmacological and toxicological properties. Secondly, current methods

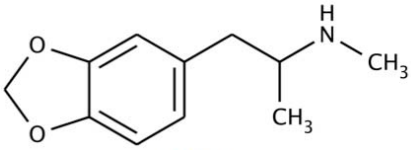
used by the governments to obtain information on population drug use are discussed, focusing on their advantages and disadvantages. Next, the method of wastewater analysis is introduced together with drug metabolic and pharmacokinetic profiles. Finally, the issues and potential applications of the wastewater analysis method are discussed, which leads to the research aims of this thesis. The illicit stimulants discussed in this thesis are confined to cocaine, methamphetamine, MDMA and some of the most reported novel synthetic stimulants (chemical structures displayed in Figure 1).



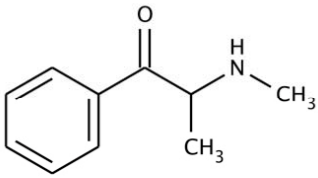
Cocaine



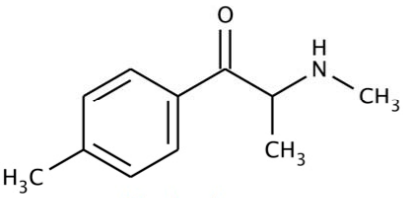
Methamphetamine



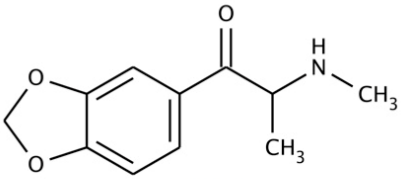
MDMA



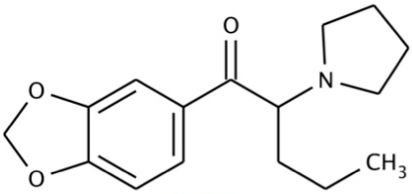
Methcathinone



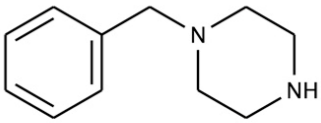
Mephedrone



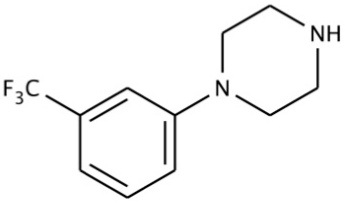
Methylone



MDPV



BZP



TFMPP

Figure 1. Chemical structures of stimulants studied in this thesis

1. Historical and contemporary stimulant abuse

1.1. Cocaine

Although cocaine was discovered in the 19th century, the leaves of *Erythroxylon coca*, from which cocaine is isolated, are believed to have been used by South American indigenous people since the 6th century A.D. or even earlier (Petersen 1977). The coca leaves were chewed as a tonic to enhance people's working ability under arduous conditions, and also as an anaesthetic. In the 19th century, cocaine isolated from coca leaves was first used as a local anaesthetic for eye, nose and throat surgery (Barash 1977). However, due to its psychoactive and reinforcing effects, cocaine was also used recreationally (Siegel 1977). Although fears of its addictive properties among the public had existed for some time, the direct cause for the first restriction of its distribution occurred in the United States in the period 1900–1920 when some crimes associated with cocaine use took place. Later, cocaine and coca use was further restricted in the Harrison Narcotics Act of 1914 (Petersen 1977). Now cocaine is controlled as an illicit drug globally by the Single Convention on Narcotic Drugs of 1961 and the United Nations Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances of 1988.

The estimated percentage of annual cocaine users in 2010 ranged from 0.3% to 0.4% of the global population aged 15–64 (UNODC 2012). Bolivia, Colombia and Peru are the major countries for cocaine production, whereas North America, South America and Western and Central Europe are the major areas for cocaine consumption (UNODC 2012). From 2006 to 2010, a decrease of cocaine use was seen in the United States as a result of stricter

control of cocaine manufacture in Columbia, suggesting that Columbia was the major source of cocaine supply for North America. At the same time cocaine use in Europe was not largely affected, which was believed to be a result of an additional supplying source from Bolivia and Peru (UNODC 2012).

Some changes have been seen in the areas with conventionally high prevalence of cocaine use. In the biggest market, North America, cocaine consumption appears to be declining in recent years; while it is increasing in the second biggest market Europe (UNODC 2010). Some recent data also suggested that cocaine use in Eastern Europe, South-East Asia and Oceania is increasing. In particular, a great increase in cocaine seizures has been seen in Oceania, where a four-time increase in cocaine use was observed between the periods of 2005/2006 and 2009/2010 (UNODC 2012).

1.2. Methamphetamine

Methamphetamine is the second most (following cannabis) popular illicit drug (Cruickshank and Dyer 2009) and most widely manufactured ATS in the world. Approximately 0.4% of the population are reported to use methamphetamine at least once a year (UNODC 2011). Methamphetamine was first synthesised from ephedrine in 1893 by the Japanese chemist Akira Ogata. Ephedrine is a natural product first isolated from *Ephedra vulgaris* by another Japanese chemist Nagai Nagayoshi (EMCDDA 2009). In the 1920s and 1930s, methamphetamine and amphetamine were used for medical and paramedical purposes to treat depression and other psychological symptoms (EMCDDA 2009). During World War II, both Axis and Allied forces used methamphetamine to help

soldiers fight off fatigue and enhance alertness. In the 1950s and 1960s, restrictions on methamphetamine use started, and illicit manufacturing of this drug emerged. Methamphetamine is now listed as a Schedule 2 controlled substance according to the Convention on Psychotropic Substances of 1971 and the United Nations Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances of 1988. Although amphetamine is used recreationally in some countries, it is also used for medicinal purposes to treat attention deficit hyperactivity disorder (Berman, Kuczenski et al. 2009). In Australia there is little non-medical use of amphetamine and hence, it is not a focus of this thesis.

Ephedrine and pseudoephedrine were the traditional synthetic precursors of methamphetamine, but in recent years the illegal producers have developed an alternative synthetic pathway. This pathway starts with another precursor 1-phenyl-2-propanone (P-2-P), or even the precursors of P-2-P such as phenylacetic acid and phenyl acetate esters. Additionally, a quicker method, called the “one-pot method”, reduced the reaction time to less than 10 min. Europe accounts for the majority of uncovered clandestine laboratories (UNODC 2012), suggesting it is the major global supplier of methamphetamine. In regards to consumption, Oceania and North America are the continents with high prevalence of methamphetamine use, but use in Asia is rising (UNODC 2012).

1.3. MDMA

MDMA was first synthesised by the Merck chemist Anton Köllisch as an intermediate product when preparing methylhydrastinine, a hemostatic medication (Bernschneider-Reif,

Oxler et al. 2006). MDMA was reported to possess potent physiological and psychological effects in the 1970s, and hence was used by some psychiatrists as a psychotherapeutic tool in their practices. Later it was used recreationally in the general population and became widely known as "ecstasy" in the drug market. In 1985, MDMA was legislated by the U.S. Drug Enforcement Administration and placed on its Schedule 1 drug list (National Institute on Drug Abuse 2006). It is now also listed as a Schedule 1 controlled substance in the Convention on Psychotropic Substances of 1971 and the United Nations Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances of 1988.

Organised crime groups in Western and Central Europe have long been considered the major sources of global MDMA supply, but recently more clandestine laboratories close to the consumption markets were reported (UNODC 2010). Similar to methamphetamine, synthesis of MDMA can be achieved through various methods. Traditionally, it was based on a precursor named 3,4-methylenedioxyphenylpropan-2-one (MDP2P or PMK), but recently a new synthetic pathway starting from safrole-rich oils, which are typically sourced from South-East Asia, was observed (UNODC 2010).

According to the World Drug Report (UNODC 2012), Oceania shows the highest prevalence of MDMA use, followed by North America and Western and Central Europe. While an increasing trend of MDMA use in Europe and America was reported from 2007 to 2010, a decline was suggested in Australia during the same period.

1.4. Novel synthetic stimulants

Novel synthetic stimulants are a group of substances synthesised in laboratories with the

aim of mimicking the effects of ATS such as methamphetamine and MDMA. Chemically, these substances can be categorised as phenethylamine-based, cathinone-based, and piperazine-based analogues, etc. Some of the new synthetic stimulants were synthesised decades ago, but only recently have become popular as recreational drugs. These drugs have a variety of street names including “bath salts” and “plant food”, and are available from different sources including street dealers, retail outlets and websites (Dargan, Sedefov et al. 2011). In 2007 the availability of authentic “ecstasy” tablets in Europe decreased, with increased prevalence of “ecstasy-like” products containing greater proportions of other synthetic stimulants (UNODC 2010). This phenomenon was suggested to be a result of strategy change by the drug producers, who aimed to avoid detection by producing these “legal highs” (UNODC 2012). The distribution of these drugs was also achieved initially by non-traditional criminal networks such as online shops to avoid legal problems. However, because of the increasing popularity and the associated harm to the population, possession and distribution of many of these drugs are banned now in most countries (Advisory Council on the Misuse of Drugs 2011).

In this thesis some of the most reported new synthetic stimulants, including synthetic cathinones methcathinone (ephedrone), 4-methylmethcathinone (mephedrone), 3,4-methylenedioxy-N-methylcathinone (methylone) and methylenedioxypyrovalerone (MDPV), as well as synthetic piperazines benzylpiperazine (BZP) and 3-trifluoromethylphenylpiperazine (TFMPP), were targeted (Figure 1). Below is a brief introduction to their histories. Phenethylamine-based synthetic stimulants are not discussed in this thesis due to lower reported prevalence compared with cathinone- and

piperazine-based ones at the time when these studies were conducted.

Methcathinone was first synthesised by a group at the University of Illinois in the United States in 1928 (Hyde, Browning et al. 1928). Although it has long been used as a recreational drug in Russia, it only pervaded European and the other countries in the early 1990s (Emerson and Cisek 1993). It is now listed as a Schedule 1 controlled substance in the Convention on Psychotropic Substances of 1971 and United Nations Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances of 1988.

Mephedrone, or 4-methylmethcathinone (4-MMC), was first synthesised in 1929 (Schifano, Albanese et al. 2011). When it first emerged on the market, it was not classified as an illegal drug. Since 2009 it has become the most popular new synthetic stimulant in Europe (Schifano, Albanese et al. 2011).

Methylone was first reported in a patent by Jacob Peyton and Alexander Shulgin in 1996 as an antidepressant (Peyton and Shulgin 1996). It is also known as bk-MDMA due to the fact that it differs from MDMA by the addition of a beta-keto group.

MDPV was first reported in 1969 as a patent owned by the German pharmaceutical company Boehringer Ingelheim (Boehringer Ingelheim 1969), but gained public attention in the late 2000s when it was sold as a recreational stimulant (EMCDDA 2010c; UNODC 2012).

Piperazines were initially used as deworming agents (White and Standen 1953). As a result, BZP was first synthesised in 1944 as an antiparasitic agent (Drug Enforcement

Administration 2012), but its potential as an antidepressant drug was explored in the 1970s (Campbell, Cline et al. 1973). The first documented incident of BZP abuse was in the United States in 1996 (Austin and Monasterio 2004). Since 2000, BZP has been available on the internet in Europe (de Boer, Bosman et al. 2001). However, New Zealand was the only country to develop a significant market of BZP between 2000 and 2008 (Cohen and Butler 2011).

TFMPP was originally used as a research agent as a direct agonist of the 5-HT_1 receptor (Fuller and Mason 1981; Martin and Sanders-Bush 1982), but was later used recreationally (de Boer, Bosman et al. 2001). As a piperazine derivative, it often co-exists with its analoge BZP (Thompson, Williams et al. 2006) and other synthetic stimulants (Zuba and Byrska 2012) in tablets or capsules distributed on the market.

2. Pharmacology of illicit stimulants

Generally, the pharmacological effects of stimulants are caused by their affinity and potency on the receptors and/or transporters of neurotransmitters such as dopamine (DA), norepinephrine (NE) and serotonin (5-HT) (Farrar and Kearns 1989; Cruickshank and Dyer 2009). Specifically, their neuropharmacological and psychological effects are mainly caused by the direct activation of neurotransmitter receptors for some of the stimulants, or increased concentrations of neurotransmitters within the synaptic cleft as a result of increased neurotransmitter release from synapsis and blockage of their reuptake in the central nervous system (CNS). Peripheral effects may be due to the binding of these stimulants on neurotransmitter receptors in the peripheral nervous system, respiratory

system or circulatory system, or as consequences of their actions on CNS. The DA pathway is the major one to be associated with addictive effects of the drugs (Blum, Chen et al. 2012). Depending on the potency and selectivity on the receptors and transporters, and whether there are other mechanisms involved, the pharmacological effects vary across the stimulants. A more detailed introduction of the mechanisms and effects of the stimulants studied in this thesis can be found in the following parts of this section.

2.1. Cocaine

Cocaine blocks the reuptake of DA, NE and 5-HT in the CNS, which increases their availability in the synaptic cleft (Harris and Baldessarini 1973; Taylor and Ho 1978; Gawin, Kleber et al. 1989). Acute neuropharmacological effects typically include the desired ones such as euphoria, elevated energy, motor excitement, talkativeness, increased sexual interest and inflated self-esteem (Carrera, Meijler et al. 2004) as well as the side effects anxiety, panic, anorexia and suicidal or homicidal behaviours (Tarr and Macklin 1987). Acute cocaine use also affects cardiovascular and respiratory systems, resulting in symptoms such as hypertension and tachypnea (Farrar and Kearns 1989).

Commonly observed side effects of chronic cocaine use include but are not limited to: reinforcement (addiction) primarily caused by the inhibition of dopamine reuptake (Cadet, Ali et al. 1995); sleep difficulties caused by the blockage of 5-HT reuptake (Tarr and Macklin 1987); epileptic seizures as a consequence of cocaine-induced hyperpyrexia (Carrera, Meijler et al. 2004) and complications in the cerebral vascular and cardiovascular systems mediated by the cocaine action on noradrenergic neurotransmission (Carrera,

Meijler et al. 2004). Chronic use of cocaine can also change the dopaminergic pathway in the brain and cause psychiatric complications (Gawin and Ellinwood 1988).

2.2. Methamphetamine

Methamphetamine is a chiral compound. The S(+) enantiomer shows significantly higher psychoactive potency than the R(–) enantiomer. The R(–) enantiomer exists in the racemic mixture with little psychoactive properties (Schep, Slaughter et al. 2010).

Methamphetamine is an indirect agonist at DA, NE and 5-HT receptors (Cruickshank and Dyer 2009). *In vitro* tests showed that (+)-methamphetamine also releases NE, DA and 5-HT from the synapsis, whith higher potency in realeasing NE (Rothman, Baumann et al. 2001). In addition, methamphetamine attenuates metabolism of these neurotransmitters by inhibiting monoamine oxidase (Sulzer, Sonders et al. 2005). These three effects altogether activate the dopaminergic circuits, noradrenergic regions and 5-HT neurons in the CNS and cause psychoactive effects. Responses to methamphetamine at or below the typical dose (30 mg) include subjective feelings of arousal, euphoria, positive mood, reduced fatigue and appetite, and short-term improvement in cognition. Users of higher doses or frequent users may suffer from psychiatric disorders such as aggressive thoughts or behaviours, even in individuals without histories of psychosis (Griffith, Cavanaugh et al. 1972). Severe psychological effects of methamphetamine can also lead to fatal accidents and homocidal or suicidal behaviours (Logan, Fligner et al. 1998; Shaw 1999; Zhu, Oritani et al. 2000).

Physical changes after methamphetamine intake include pupil dilation, heart rate acceleration, blood pressure elevation and body temperature increase, etc. (Cruickshank and Dyer 2009). The mechanism of pupil dilation is via contraction of the dilator muscle of the iris caused by α1-adrenoceptor stimulation (Fotiou, Fountoulakis et al. 2000). Increased heart rate and blood pressure are caused by activation of β-adrenoceptors and α-adrenoceptors in cardiac tissue, respectively (Simpson 1975), but can also be a result of activated noradrenergic pathways, which involve the forebrain and brainstem (Crick, Sheppard et al. 2000). Severe cadiovascular and respiratory side effects can even lead to fatalities (Inoue, Ikeda et al. 2006).

2.3. MDMA

MDMA releases DA, NE and predominatly 5-HT from the synapsis (Cole and Sumnall 2003) and suppressing their metabolism (Leonardi and Azmitia 1994), which increase the extracellular concentrations of these neurotransmitters. MDMA also leads to increased excretion of cortisol (Connor, McNamara et al. 1998), oxytocin (Wolff, Tsapakis et al. 2006) and ghrelin (Kobeissy, Jeung et al. 2008), and decreased release of leptin, growth hormone and neuropeptide-Y (Kobeissy, Jeung et al. 2008), which may also contribute to its psychological effects.

Acute psychoactive effects of MDMA, which make it popular include: euphoria, positive thoughts, happiness, calmness, relaxation and heightened sensory awareness. In particular, MDMA enhances the feeling of “closeness” to others, hence is found helpful for socialisation in rave parties and dance clubs (Green, Mechan et al. 2003). Aggressive

behaviours, which are often seen in other stimulant users, are not common among MDMA users.

MDMA has side effects on cardiovascular, respiratory, urinary and peripheral nervous systems, resulting in symptoms including hypertension, pulmonary congestion, urinary urgency and muscle aches. (McCann, Slate et al. 1996; Green, Mechan et al. 2003). Psychological side effects of MDMA can persist long after cessation of drug use. These effects include visual hallucinations, paranoid delusions, cognitive impairment, anxiety, panic disorder, depression and other behavioral changes. Regular MDMA use can even lead to psychosis (Creighton, Black et al. 1991; McCann and Ricaurte 1991; Bolla, McCann et al. 1998; Parrott and Lasky 1998; Morgan 1999; Bhattachary and Powell 2001). It is widely recognised that MDMA is a less addictive drug than many of the other stimulants (Koesters, Rogers et al. 2002). However, there are some cases in which syptoms of dependence were seen (Jansen 1999).

Hyperthermia is one of the major MDMA-induced toxic symptoms, which may lead to fatalities associated with rhabdomyolysis, disseminated intravascular coagulation and acute renal failure (Green, Mechan et al. 2003). Activation of the hypothalamic-pituitary-thyroid/adrenal axis and the sympathetic nervous system plays a role in this phenomenon (Mills, Rusyniak et al. 2004), but the mechanisms are not fully understood.

A major consequence of long-term MDMA use is cerebral 5-HT loss. Positron emission tomography scans suggested that the density of 5-HT transporter sites in the brain of

MDMA users are lower than that of non-users (McCann, Szabo et al. 1998; Ricaurte, McCann et al. 2000). Neuroendocrine test, which is conducted to assess 5-HT synthesis and release, also suggested that this function was blunted in MDMA users with reduced activity of postsynaptic 5-HT_{1A} and 5-HT_{2A}/5-HT_{2C} receptors (Gerra, Zaimovic et al. 1998).

2.4. Novel synthetic stimulants

Due to the similarity of synthetic cathinones to methamphetamine and MDMA in chemical structure, similar mechanisms and effects are expected. This assumption is supported by animal tests as well as user reports: rats trained to recognise methamphetamine and MDMA also act on synthetic cathinones (Dal Cason, Young et al. 1997); desired effects of cathinones reported by users are similar to methamphetamine and MDMA, which include increased energy, openness and elevated libido (Winstock, Mitcheson et al. 2011). The potencies of the cathinones can be better understood when compared with cocaine, methamphetamine and MDMA. *In vitro* tests suggested that methcathinone and methylone inhibited catecholamine transporters with a similar potency as their respective analogues methamphetamine and MDMA (Cozzi, Sievert et al. 1999). Mephedrone inhibits DA transporter to the same extent as methamphetamine (Cameron, Kolanos et al. 2013). When compared with MDMA, mephedrone showed less potency in increasing 5-HT concentration in the brain, but higher potency in increasing DA levels (Kehr, Ichinose et al. 2011). *In vitro* (Cameron, Kolanos et al. 2013) and *in vivo* (Baumann, Partilla et al. 2013) tests of MDPV both indicated that it is a non-substrate monoamine transporter blocker with

much higher potency and selectivity for catecholamines when compared with cocaine.

For synthetic piperazines, *in vitro* tests revealed that BZP displays strong inhibition of DA and NE reuptake, but only slightly affects 5-HT reuptake (Nagai, Nonaka et al. 2007). Animal tests also suggested that BZP displays sensitisation and cross-sensitisation to methamphetamine at a 10 times higher dose (Brennan, Johnstone et al. 2007). However, as a selective 5-HT_{1b} receptor agonist, TFMPP displays psychedelic effects like the other 5-HT receptor agonists lysergic acid diethylamide (LSD) and MDMA, and the potency was reported to be between the two (Antia, Tingle et al. 2009). Consequently, BZP and TFMPP are often mixed (at a ratio of 2:1 in many cases) in illicit pills to give a combining effect similar to MDMA (de Boer, Bosman et al. 2001). Oral administration of 100 mg BZP and 30 mg TFMPP by healthy adult men increased blood pressure and heart rate, and gave a subjective feeling of increased activity and dysphoria (Lin, Jan et al. 2011). The long term effects of chronic use of the novel synthetic stimulants are not known, but are expected to be similar to the traditional stimulants.

3. Social burden of illicit drug use

Among the 7 billion people in the world, approximately 230 million, which equals to 1 in 20 persons between 15 and 64 years old, use illicit drugs at least once a year. 1 in 40 of the same group of people use drugs at least once a month (UNODC 2011). It was also estimated that the total value of the illicit drug market for 2003 was 320 billion USD, which was equivalent to 0.9% of the global GDP (UNODC 2005).

Currently, illicit drug use in the population is concentrated among youth in urban areas, and there are more male users than females. The reason for the higher prevalence of illicit drug use among young people may be that this group of people are more tempted to transgress laws and social norms, rather than being more susceptible to the psychoactive effects of the illicit drugs. This hypothesis is supported by the fact that the use of legal psychoactive substances (tobacco and alcohol) does not decrease as quickly as illicit drugs from adolescence to maturity (UNODC 2012).

Use of illicit drugs, including stimulants, can have great impact on the society. This impact can be categorised into three aspects, namely health-related issues, crimes and social capital costs.

3.1. Health-related issues

Drug use can have a serious negative impact on the health of the users, even if their use is occasional. The most severe consequence is death related to drug-induced accidents, fatal behaviours associated with drug use, drug overdose, or severe medical conditions caused or exacerbated by illicit drugs. This is particularly true for young people, as the mean age of deaths related to drug overdose is in the mid-30s in Europe (EMCDDA 2010a). World Health Organisation (WHO) estimated that ATS, cocaine and heroin together caused 0.2 million (0.4% of the population) global deaths (WHO 2002). The side effects of illicit drugs may also cause severe health problems to the users, particularly when drug addiction develops and high doses are used, as discussed in Section 2 of this chapter. In the same report mentioned above (WHO 2002), it was estimated that 11.2 million (0.8% of the total)

of "disability-adjusted life-years" were lost due to the use of illicit drugs.

Injecting drug users also face the risk of transmitting diseases including AIDS and hepatitis B and C. Among the 16 million injecting drug users in the world, one in five is human immunodeficiency virus (HIV)-positive as estimated by UNODC (2012). About the same percentage of injecting drug users are infected with hepatitis B, and half of them are hepatitis C carriers (UNODC 2012). These diseases can cause severe negative effects or even deaths.

3.2. Crimes

Illicit drug use is also associated with crimes. UNODC (2012) estimated that drugs accounted for approximately 20% of global criminal proceeds. These crimes can be commited by both users and traffickers. The involvement of illicit drug users in crimes may be a result of their abnormal psychological status after the intake of psychoactive drugs, or driven by their demand to finance the habit. For example, urine tests in the United States in 2010 revealed that 70% of arrested males had used an illicit drug (Abt Associates Inc. 2011). A similar study in Australia also found that 65% of those detained for criminal activity were tested positive for illicit drugs (Gaffney, Jones et al. 2010). Illicit drug trafficking is a crime in itself in most countries, and can escalate criminality since drug trafficking is believed to be a major financial source of organised criminal groups. For instance, when there is competition of market share between two or more trafficking groups, violence including homicide may occur as is currently happening in countries such as Mexico (Shirk 2011). Drug dealers may also encourage corruption in political and

government authorities (UNODC 2012).

3.3. Social capital costs

Social capital cost is a subsequent consequence of health-related issues and crimes caused by illicit drug use. The loss associated with health issues can be further divided into two parts, namely treatment costs and potential productivity loss.

UNODC estimated that among all illicit drug users, about 12% develop dependency and become "problem" ones (UNODC 2011). Specifically, 15% of cocaine users are dependent (American Psychiatric Association 1994), and this percentage increases to 26% for methamphetamine (UNODC 2012). Medical treatment is required to help these users, which generates financial burdens to the person, their families and the whole society. Nevertheless, not all dependent users have received the medical treatment that they need. For instance, while 7.9 million users in the United States required treatment in 2010, only 2.2 million received it (Substance Abuse and Mental Health Services Administration 2012). It is estimated that with the current drug prevalence, 200–250 billion USD (0.3–0.4% of the global GDP) would have been required to adequately deal with this issue (UNODC 2012).

Potential productivity loss is calculated as the value of work that has not been performed due to illicit drug use. Using this method, it was estimated that 120 billion USD (0.9% of GDP) was lost in the United States in 2007 due to illicit drug use, which represented 62% of all drug-related costs. (United States Department of Justice and National Drug Intelligence Center 2011). Similar studies in Canada and Australia showed that the

percentage of GDP loss due to illicit drug use was 0.7% and 0.88%, respectively (Rehm, Baliunas et al. 2006; Collins and Lapsley 2008).

Crimes associated with illicit drugs cost social capital in a variety of ways, including policing, criminal courts, prisons, customs, property theft and damage and money laundering (Collins and Lapsley 2008). In England and Wales, a total estimation of £13.9 billion, which equates to 90% of all social and economic costs related to drug abuse, was caused by drug-related crimes in 2003/2004 (Gordon, Tinsley et al. 2006).

In summary, illicit drug use has a significant negative impact on the society, which is characterised by impaired health, increased crimes and wasted social capital. Reducing this negative impact is a challenging obligation for all authorities.

4. Current monitoring methods

4.1. Significance of drug control policies

To minimise the negative impact of drug use on the community, it is essential to reduce the number of illicit drug users in the population by controlling the production, trafficking, possession and consumption of the drugs. Legislations aim at reducing the number of drug producers and traffickers who are prepared to run the risks, hence limits the amount of illicit drugs available on the market. Law enforcement also keeps potential users away from the illicit drugs and encourages regular users to quit the habit and seek treatment (UNODC 2012). Additionally, the risk associated with the production and trafficking elevates the prices of the illicit drugs. While the retail weight price of cocaine and heroin

might be as low as coffee if they were not under control, the actual prices in the black market are higher than gold (MacCoun and Reuter 2001). This elevated price has an important impact on users' habit of consumption. It was found that although addicted drug users continue to use drugs when the price goes up, the long-term consumption will eventually decrease due to their difficulties in financing the habit. On the other hand, addictive users increase their use easily when the price falls. Recreational drug users, however, react quicker to the price in both directions (Abt Associates Inc. 2001). Hence, driving up the price of illicit drugs and keeping it at high levels as a consequence of drug control may effectively reduce consumption of illicit drugs by both addictive and recreational users. From an economic point of view, for every dollar spent on alcohol and drug control, seven dollars of social capital are saved, mostly on crime and health care costs (Hatch 1997).

The first national-level control of drug use was the prohibition of non-medical opium use in the 19th century in China. However, the opium merchants and trade were supported by the government of the British Empire at that time due to the high profits. This conflict of interest eventually turned into the Opium Wars. China lost the wars and was forced to open up for the opium trade. By the early 20th century, it was estimated that about a quarter of male adults in China used opium (International opium commission 1909). Similarly, the state-level control of cocaine in the United States and the nation-wide ban of hashish by Egypt in the 20th century both failed, as drugs were brought in from neighbouring states or countries (UNODC 2012). Soon after that it was widely recognised that national-level effort was not sufficient in controlling drug use, and a number of international acts were

announced consequently. The first international conference on drugs control was held in Shanghai, China in 1909. Three year after that the adoption of the International Opium Convention was singed. In 1961, 1971 and 1988, three international conventions took effect, which were the Single Convention on Narcotic Drugs, Convention on Psychotropic Substances, and the United Nations Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances, respectively. These United Nations conventions are widely adhered to and have formed the foundation of the contemporary international drug control systems.

4.2. Significance of information on illicit drugs

Information on the illicit drug use in the population is essential to improve the cost-efficiency of policies and healthcare resources spent to reduce the harm related to this issue. Additionally, monitoring the change of population illicit drug use during or after an intervention is required to assess its impact and refine programs to improve outcomes (Saxena and Donoghoe 2000).

Illicit drug use is a global phenomenon, and hence many countries share some similar characteristics in population drug use. For example, illicit drug use is concentrated in urban settings among young males, as discussed previously; social stratification is related to drug use, with higher prevalence in the lower and upper classes and lower prevalence in the middle classes. However, some differences in population illicit drug use across different regions exist. For instance, cocaine is the preferred stimulant in America, whereas ATS are often the drugs of choice in Oceania. More recently, the impact of globalisation has been

reflected in quick changes of drug use in certain areas as a result of international trends. An example of this is the decline in cocaine in North America accompanied by an increase in South America, Western Europe and Africa in recent years (UNODC 2012). Hence, accurate and up-to-date information on community illicit drug use is needed if important sudden changes in population drug use is a common trend.

Currently, psychoactive substance use is monitored in several ways. Most of the information is collected by government agencies via surveys and reporting systems.

4.3. Surveys

General population surveys have been used as a major tool to collect information on population use of licit and illicit drugs. In the 1980s the WHO and the United Nations started to develop standardised surveys collaboratively, which provided more internationally comparable data. Two designs of surveys are usually employed, namely the single cross sectional survey and the repeated cross sectional survey. The former provides descriptive information on population drug use at a point of time, such as whether a drug or phenomenon exists, what the characteristics of the users and drugs are, and what the causes and consequences may be. In contrast, the latter survey method provides temporal comparison data to show the change of situation between each survey (Johnston 2000). Although the surveys collect a large amount of information related to drug use in the population, the method is very resource intensive. For example, the average cost to complete an interview, which does not include data analysis and resources such as office facilities, has already reached the range of 300–500 USD in North America (Johnston

2000). Aside from the costs, other critical issues also exist: it is often difficult to have a face-to-face interview with a respondent due to consideration of time and privacy, particularly for remote areas and marginalised population groups; bias due to cultural or political views can occur in the interaction between the interviewer and respondent (EMCDDA 2002).

In Australia, a series of national surveys named National Drug Stratagy Household Surveys (NDSHS) are conducted every three years, in which a large number of staff and a great amount of resources are involved. These surveys provide cross sectional data on the population use of alcohol and other drugs, residents' attitudes towards drug use and community support for drug-related policies (Australian Institute of Health and Welfare 2008). A comprehensive report is published online after each survey. However, this report when published normally refers to data collected one year previously due to the time required for data analysis (Australian Institute of Health and Welfare 2011).

4.4. Reporting systems

Reporting systems serve as supplementary information sources of surveys for gathering information on population drug use. These systems include event reporting, case reporting, and case registries. Apart from regular users, information is often collected from agencies such as customs, police and hospitals (Smart 2000).

Illicit drugs seized by customs and police are resources for evaluating the demand for these psychoactive substances in a particular country. These agencies regularly publish seizure reports on illicit drugs (Australian Federal Police 2004; Australian Crime Commission

2009). It is possible to obtain some epidemiological information by analysing these data (Knolle 1999). However, this gives little indication on where exactly the drugs are sold and consumed. Moreover, the seized illicit drugs are only a proportion of the total amount, thus it is unlikely to accurately reflect the prevalence of drugs in a nation (Jiggens 2008). There is also a possible bias when interpreting supranational data because of differences in efficiencies in seizing illicit drugs across countries.

In addition to routine seizures, police may also conduct random drug tests with the purpose of preventing crimes and offences associated with illicit drug use. For example, South Australian Police carries out roadside drug testing in the hope of preventing drug-affected driving and reducing the number of fatalities on roads. Saliva samples of randomly selected drivers are collected and tested beside the road for popular illicit drugs (currently tetrahydrocannabinol (THC), methamphetamine and MDMA) using fast-diagnostic kits. Positive samples are then sent to the local forensic science centre for confirmation. The number of positive tested drivers provides prevalence data of the targeted drugs (Chen, Kostakis et al. 2011). However, the sample size is restricted to drivers, and the tests are often conducted at times and places with which illicit drug use is expected to occur (e.g. weekend nights near entertainment districts). As a result of these sampling strategies, the sample size does not represent the whole population. Moreover, these tests only indicate whether the driver has or has not used drugs, regardless of the dose consumed.

Hospitals are other institutions which often deal with drug issues. Patients with illicit drug problems are good statistical samples. Their home addresses are also useful information

sources for analysing drug use in certain regions. However, the patients are likely to underreport their use of illicit drugs, thus reducing the reliability of these data (Chen, Fang et al. 2006). Medical staff's priority is the immediate health of the patient and thus collection of drug information is secondary and often sporadic and incomplete. Moreover, users of low-dose drugs who do not require treatment for severe side effects are not included in this database.

There are a number of specialised reporting systems for monitoring the use of illicit drugs and alcohol in Australia. They are known as Illicit Drug Reporting System (IDRS), Ecstasy and Related Drugs Reporting System (EDRS) and National Illicit Drug Indicators Project (NIDIP) (NDARC 2007).

IDRS is a system for monitoring the purity, price, availability and use patterns of heroin, cocaine, cannabis and methamphetamine in Australia. It also identifies trends in illicit drug use that require further investigation. It collects and analyses three sources of data, namely (1) interviews with regular injecting drug users; (2) semi-structured interviews with professionals who work with drug users; (3) existing databases on drug-related issues. By combining these three data sources, IDRS minimises the weaknesses of each one, and simply records emerging trends in drug use (NDARC 2007). However, the system cannot decipher the general population pattern of drug use and the size of the drug market (Stafford 2009).

The EDRS, formerly known as the Party Drug Initiative (PDI), focuses on the use and markets of ecstasy and related drugs in Australia. The targeted drugs include

methamphetamine, γ-hydroxybutyrate (GHB) and ketamine. Similar to the IDRS, the EDRS is also designed to identify trends that require further research (NDARC 2007). Its advantages and weak points are also similar to those of IDRS (Stafford 2009).

The aim of NIDIP is to supplement the IDRS and EDRS by standardising and evaluating the indicator data collected, and to translate the indicator data in a more accurate and timely way (NDARC 2007). NIDIP deals with a large amount of data from national and jurisdictional-based data collection systems. The problems of this project include the incompleteness of data, poor comparability and the lag-time involved in data collection (NDARC 2009).

4.5. International reports

Integrated worldwide information on illicit drug use is essential for international cooperations to control the problem. The annual World Drug Reports published by the United Nations Office on Drugs and Crime (UNODC) aims to develop an integrated approach and assist member states in reducing illicit drug use. The approaches include integrated regional programs as well as inter-regional and inter-agency systems. The reports present recent situations of the global illicit drug markets and problems associated with them, including market scale, characteristics, patterns and driving factors (UNODC 2012).

The World Health Organisation (WHO) also publishes reports related to the global issues of illicit drugs, but focuses more on the prevention and treatment side with the aim of improving the coverage and quality of these interventions for substance use disorders

(WHO 2010).

In Europe, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is the organisation to gather and analyse information on illicit drug use. Annual reports as well as quarterly newsletters (Drugnet Europe) are both available online. The data are collected via collaboration with a number of agencies in Europe (EMCDDA 2012).

4.6. Summary

The above approaches for monitoring illicit drug and alcohol use are mainly based on surveys and reporting systems. They provide a snapshot of information on illicit drug use in the population at a given time point. An important aspect of the information gathered by these approaches is the users' profiles (social-economic status, age, gender, education, etc.), which can be linked to their drug use habit. Analysis of such information enables professionals find out which factors have significant impact on illicit drug use, and assist authorities to target subgroups in the population with a higher prevalence of illicit drug use. These data sources also provide valuable information on the price, availability and route of administration of the illicit drugs.

There are disadvantages with these conventional systems, too. Firstly, the surveys and users' reports heavily depend on subjective responses of illicit drug users. Intentional and unintentional underreporting or overreporting are possible among users, particularly when users are not familiar with the illicit drugs they consume (Wood and Dargan 2012). Secondly, there may be sampling issues related to these methods, such as difficulties in assessing remote areas and marginalised groups, and non-representative sampling in

reporting systems of people who are more susceptible to the targeted illcit drugs. Thirdly, gathering the information is expensive in terms of labour, funding and time (Johnston 2000). The lengthy delays in reporting also limits its value in a rapidly changing drug scene. Additionally, it is important to have international comparisons (Saxena and Donoghoe 2000), but this is not easy with these methods when non-standard questionnaires are used. The reporting systems may also vary across countries.

Based on the facts mentioned above, it is clear that information on illicit drug use in the population is essential to assist authorities protect the community by reducing the associated negative impact. However, current monitoring methods are far from adequate, due to their inherent disadvantages that include inaccuracies, slowness, resource-dependence and lack of comparability. This is particularly the case for the modern world where new drugs are emerging on a daily basis. Hence, a new method which can serve as an alternative or a supplementary information source is urgently required to provide more comparable data in an accurate, cost effective and near real-time way.

5. Wastewater epidemiology approach

5.1. Origin

In 2001, Daughton (2001) proposed a novel method, in which advanced analytical technologies are used to identify and quantify the illicit drugs in the influents of wastewater treatment plants (WWTPs). The illicit drugs detected are either from human excretion or from clandestine lab disposal. He suggested that this method can serve as an inexpensive way to obtain objective data on the manufacturing, disposal, and consumption

of illicit drugs in a community in an indirect but objective way.

This proposal was first realised in 2005 by a group led by Zuccato (2005), although detection of illicit drugs in wastewater samples had been achieved prior to that (Jones-Lepp, Alvarez et al. 2004). In the study conducted by Zuccato et al, river water samples and wastewater samples from WWTPs of medium-size Italian cities were analysed by liquid chromatography coupled with mass spectrometry (LC-MS), targeting cocaine and its metabolite benzoylecgonine. The results revealed the presence of these two compounds. The authors then back-calculated the cocaine consumption based on the measured concentration of benzoylecgonine, the water flow rate and the census population. It was suggested that the estimated cocaine consumption in this study was much higher than those reported in surveys. Later, this method was developed and employed in other countries, often under the name of "wastewater epidemiology" (van Nuijs, Mougel et al. 2011).

5.2. Metabolic and pharmacokinetic foundations

According to the rationale of the wastewater epidemiology approach, a good knowledge of the metabolism and pharmacokinetics of the illicit drugs of interest is essential. Knowing the metabolism of the drug helps determine which metabolite (or the unchanged parent drug) is a suitable analytical target; while the pharmacokinetic properties of an illicit drug can suggest the lag-time between drug consumption and excretion, which helps determine the represented time of drug consumption for each collected wastewater sample.

Ideally, an analytical target used in the wastewater epidemiology study should meet three criteria. Firstly, it should be an exclusive metabolite of the drug of interest. Being a

metabolite excludes the fraction of drugs directly dumped into the wastewater system without consumption by humans, and being an exclusive one reduces the interference from the consumption of other drugs in the population. Secondly, the excretion ratio (the mass excreted as the metabolite ratios the mass of the parent drug) should be relatively high and constant, thereby allowing easy detection of the analytical target and reliable back-calculation. Thirdly, the metabolite needs to be stable and should not bind to particulate matter in wastewater or the filter paper during sample preparation. The reasons for the third criterion are discussed in more detail in Section 6.1 of this chapter.

Nevertheless, it is not always possible to meet all the three criteria and sometimes compromises have to be made. For drugs such as methamphetamine that are not extensively metabolised (Cook, Jeffcoat et al. 1993), the parent drug is normally used as the analytical target, allowing the possibility of interference from unused drug disposal down the drain. Another example which exhibits the complexity of choosing an analytical target is heroin. When consumed, heroin is quickly metabolised in the liver and eliminated from the body as morphine and morphine-glucuronide. The latter is further hydrolysed to morphine in the wastewater by bacteria (Castiglioni, Zuccato et al. 2008). Hence, when solely considering the metabolism of heroin, morphine seems to be the best analytical target. However, consumption of morphine, codeine and possibly other opioids can also result in morphine excretion, hence the measured morphine in wastewater samples does not directly reflect heroin consumption in the population. To cope with this issue, a minor but exclusive metabolite of heroin, 6-monoacetylmorphine, may be a more suitable analytical target for heroin use estimation (Castiglioni, Zuccato et al. 2006), even though

its concentration (van Nuijs, Tarcomnicu et al. 2009) and stability (Castiglioni, Zuccato et al. 2006; van Nuijs, Abdellati et al. 2012) in wastewater are low. Thus, when choosing an analytical target, a number of factors, including the metabolism of the drug of interest and other related drugs need to be taken into consideration. This section summarises the metabolic and pharmacokinetic properties of the stimulants studied in this thesis where such information is available, and links this knowledge to the purpose of this thesis.

5.2.1. Cocaine

The half-life of cocaine is approximately 0.6 hours after intravenous administration, 0.9 hours if orally taken, and 1.3 hours for nasal intake (Van Dyke, Jatlow et al. 1978; Javaid, Musa et al. 1983). Cocaine is rapidly metabolised in the liver, mainly into the forms of benzoylecgonine and ecgonine methyl ester, and renally excreted. Elimination of benzoylecgonine is prompt and greater if the urine is acidic (Carrera, Meijler et al. 2004). Cocaine can also be metabolised into other compounds such as norcocaine in smaller proportions (Carrera, Meijler et al. 2004) (Figure 2).

Since benzoylecgonine is the major metabolite of cocaine, it has been used as the analytical target for cocaine use monitoring in wastewater analysis. Cocaine and other metabolites are also analysed but were found less suitable than benzoylecgonine due to lower concentrations and stability in wastewater (Gheorghe, van Nuijs et al. 2008). The relatively short half-life of cocaine also indicated that a wastewater sample collected in a 24-hour session largely reflects drug use on the sampling day.

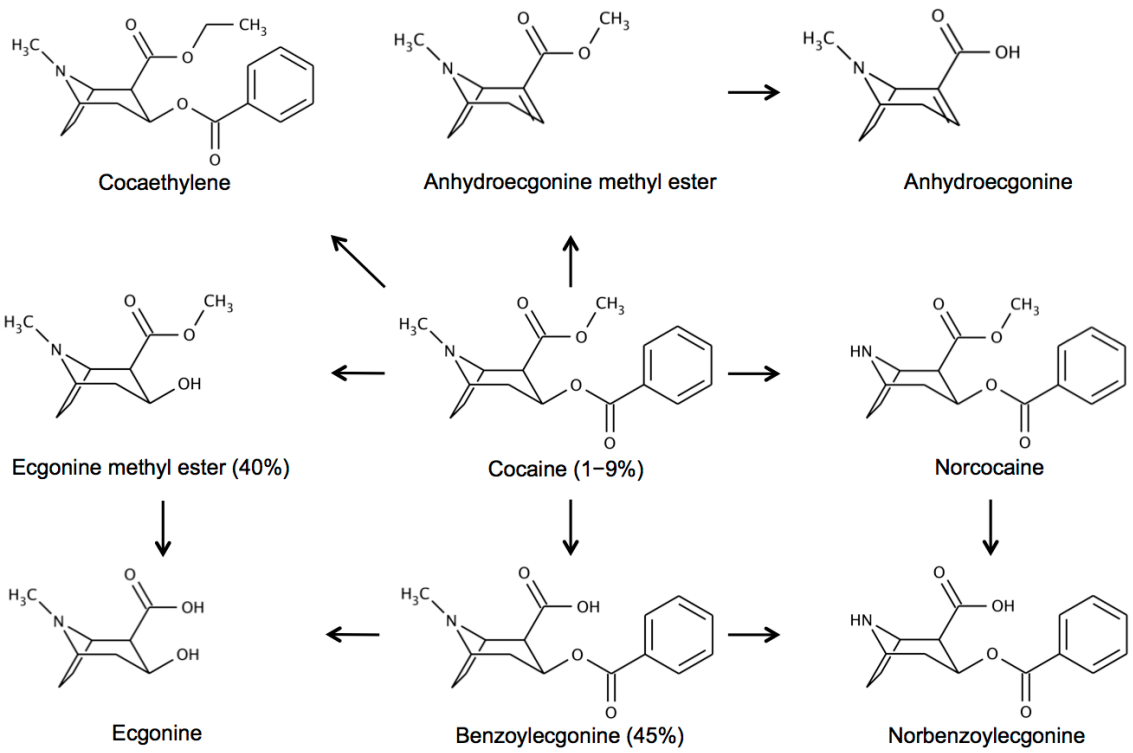


Figure 2. Metabolic pathways of cocaine in humans, with percentages of excretion in the urine (derived from Castiglioni et al. 2008)

5.2.2. Methamphetamine

Bioavailability of methamphetamine is at or above 67% when smoked or orally taken, and can reach 79% if nasally administered. Methamphetamine is mainly excreted in the urine unchanged (Cook, Jeffcoat et al. 1993), with a small proportion metabolised in the liver to amphetamine or 4-hydroxymethamphetamine, in which cytochrome P450 2D6 is involved, or to norephedrine via β-hydroxylation (Caldwell, Dring et al. 1972). The proportion of each metabolite can vary because of the polymorphic cytochrome P450 2D6 (Lin, Di Stefano et al. 1997), but does not appear to be affected by chronic use (Cook, Jeffcoat et al. 1992). Other minor metabolites of methamphetamine such as N-hydroxymethamphetamine (Baba, Yamada et al. 1987), 4-hydroxyamphetamine (Caldwell, Dring et al. 1972) and

N-hydroxyamphetamine (Baba, Yamada et al. 1987) have also been reported (Figure 3). Khan and Nicell (2012) summarised clinical metabolic studies on methamphetamine, and found that the proportion of methamphetamine excreted in the urine within 48 hours after intake (regardless of dose and route of administration) ranged from 36.9–52%, and there was no difference between S(+) and R(–) methamphetamine in the proportion of methamphetamine excretion after oral ingestion. The above information suggested that methamphetamine itself as a racemate is the most suitable analytical target for wastewater epidemiology studies. Castiglioni et al suggested an excretion ratio of 43% (2008), which is within the range suggested by Khan and Nicell (2012) and applied in this thesis.

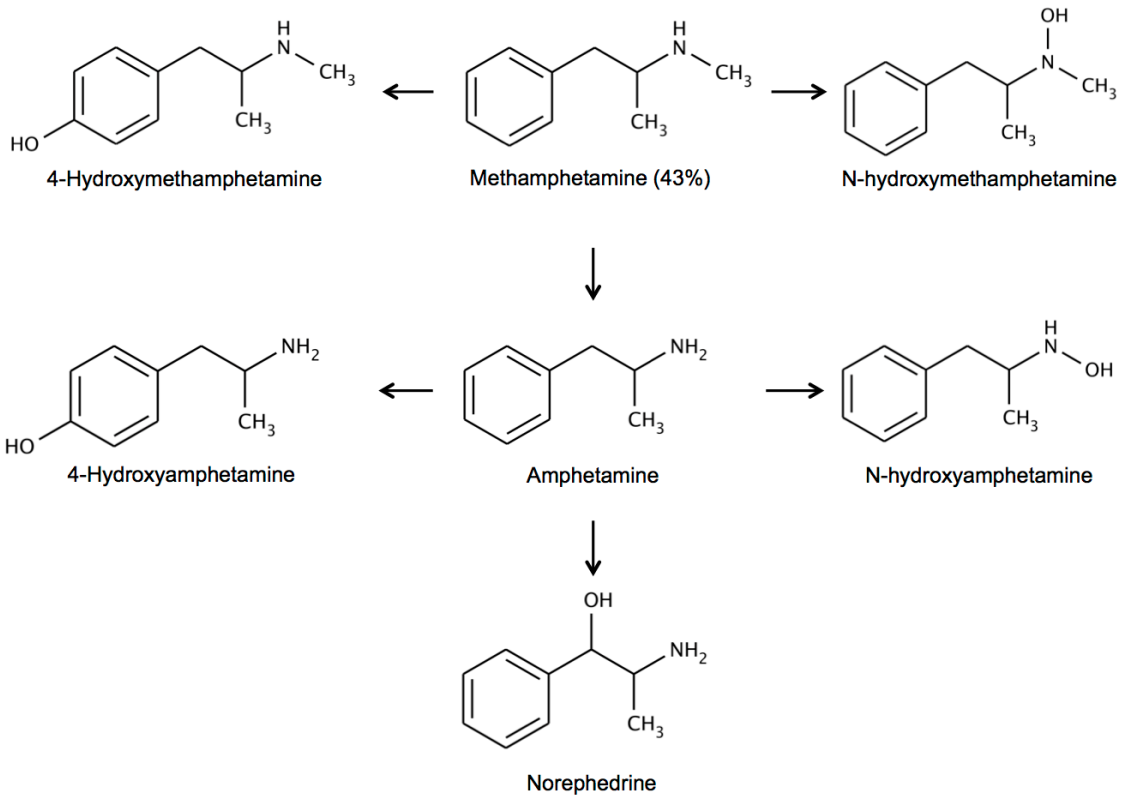


Figure 3. Metabolic pathways of methamphetamine in humans, with percentage of excretion in the urine

The plasma half-life of methamphetamine is approximately 10 hours, but it can vary

substantially among individuals (Cruickshank and Dyer 2009). After a single dose, methamphetamine is detectable in the urine for up to 60 hours, but is mostly eliminated from the human body within 24 hours after consumption (Cook, Jeffcoat et al. 1993). These data indicate that a 24-hour composite wastewater sample mainly reflects methamphetamine use on that day.

5.2.3. MDMA

MDMA is metabolised into a variety of compounds via different pathways. It can be ring-hydroxylated to 2-hydroxy-4,5-methylenedioxymethamphetamine (6-OH-MDMA), N-demethylated to methylenedioxyamphetamine (MDA), and O-demethylated to 3,4-dihydroxymethamphetamine (HHMA, DHMA) (Lim and Foltz 1988; Lim and Foltz 1991a; Lim and Foltz 1991b). MDA can be further O-demethylated to 3,4-dihydroxyamphetamine (HHA, DHA). HHMA (DHMA) and HHA (DHA) can be metabolised into other compounds with toxic and psychological effects, which are believed to contribute to the pharmacological and neurotoxic effects of MDMA (Green, Mechan et al. 2003). One important property of MDMA is that it inhibits its own metabolism. This phenomenon results in nonlinear pharmacokinetics of MDMA after a single dose and greater increase of MDMA concentration in the blood than simple accumulation after repeated doses (de la Torre, Farre et al. 2004).

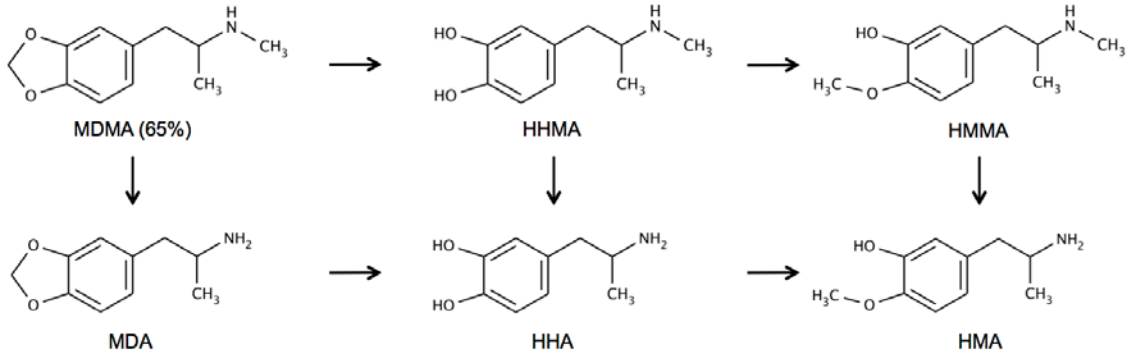


Figure 4. Metabolic pathways of MDMA in humans, with percentage of excretion in the urine (derived from de la Torre et al 2004 and Zuccato et al 2008). MDMA: 3,4-methylenedioxy-N-methylamphetamine; HHMA: 3,4-dihydroxymethamphetamine; HMMA: 3-hydroxy-4-methoxymethamphetamine; MDA: 3,4-methylenedioxyamphetamine; HHA: 3,4-dihydroxyamphetamine; HMA: 4-hydroxy-3-methoxyamphetamine

At doses from 40 to 100 mg, the excretion ratio of unchanged MDMA is in the range of 15–22% in most reported studies (de la Torre, Farre et al. 2004; Khan and Nicell 2011), which is much lower than 65% that was first reported by Verebey et al (1988) and used by Zuccato et al (2008) in the back-calculation of wastewater epidemiology studies. In this thesis we use the same excretion ratio of 65% in order to facilitate comparisons of wastewater data between Australia and European countries.

5.2.4. Novel synthetic stimulants

The synthetic cathinones are metabolised in similar pathways to methamphetamine and MDMA. This normally includes phase 1 metabolisms N-demethylation, O-methylation, demethylation of the methylenedioxy ring (if present), N-dealkylation, and oxidation or reduction, and phase 2 conjugations to form glucuronide and sulphate.

Specifically, methcathinone is metabolised to ephedrine or pseudoephedrine through reduction of the carbonyl group (Paul and Cole 2001) (Figure 5), both of which are used as

bronchodilators (Drew, Knight et al. 1978) and precursors to produce methcathinone (Paul and Cole 2001) and methamphetamine.

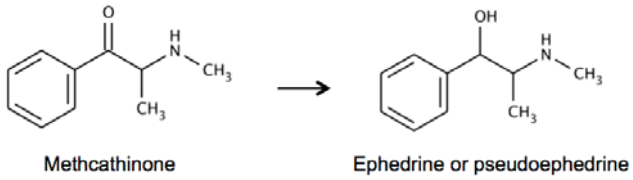


Figure 5. Metabolic pathway of methcathinone in humans (derived from Paul et al 2001)

Mephedrone is metabolised into a primary amine via N-demethylation, and then reduced to alcohol by reduction of the carbonyl group, as well as oxidation to form the toluyl group (Figure 6). Some of the metabolites are conjugated via sulfation or glucuronidation (Meyer, Wilhelm et al. 2010).

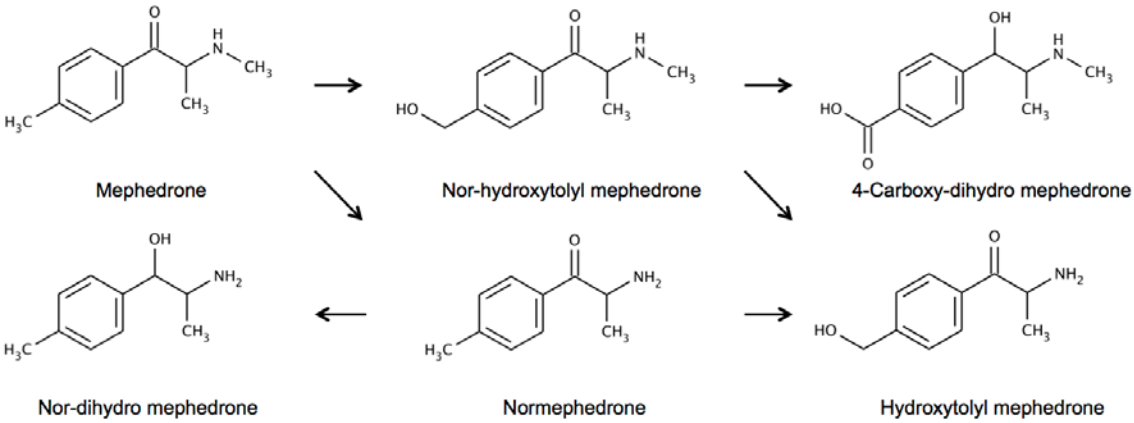


Figure 6. Proposed metabolic pathways of mephedrone in humans (derived from Meyer et al 2010)

Primary metabolism of methylone mainly involves degradation of the side chain by N-demethylation or demethylenation followed by O-methylation. 4-Hydroxy-3-methoxymethcathinone (HMMC) is the major metabolite in both humans and

rats. These metabolites are partially excreted as glucuronide and sulphate conjugates (Kamata, Shima et al. 2006) (Figure 7).

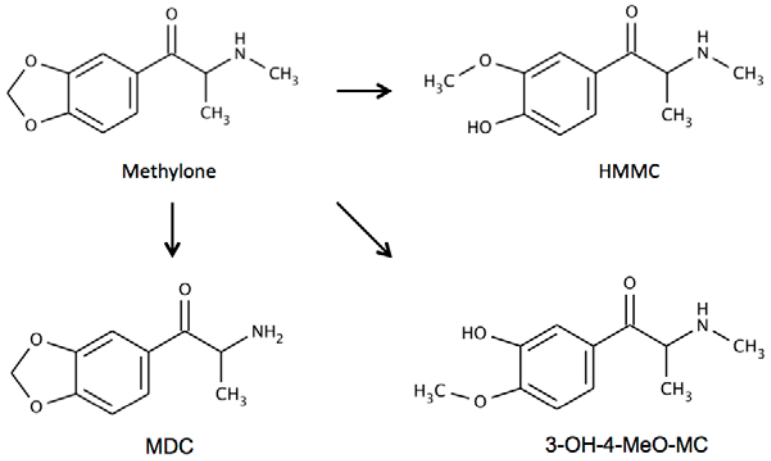


Figure 7. Proposed metabolic pathways of methylone in humans (derived from Kamata et al 2006). MDC: methylenedioxycathinone; HMMC: 4-hydroxy-3-methoxymethcathinone; 3-OH-4-MeO-MC: 3-hydroxy-4-methoxymethcathinone

Metabolic pathway of MDPV starts with breakage of the methylenedioxy ring and followed by demethylation and O-methylation to form catechol and methyl catechol pyrovalerone, then ends with sulphate or glucuronide conjugation (Strano-Rossi, Cadwallader et al. 2010) (Figure 8).

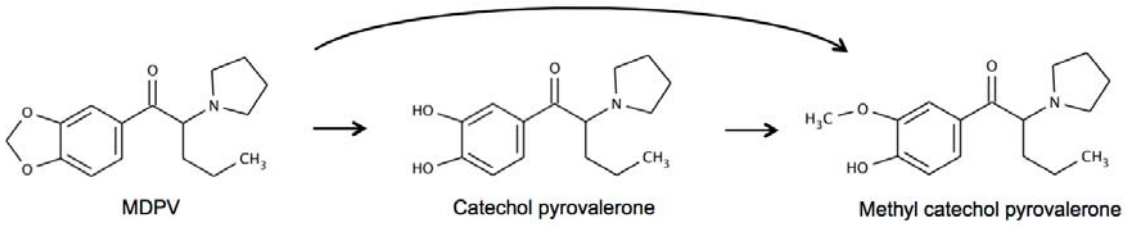


Figure 8. Proposed metabolic pathways of MDPV *in vitro* (derived from Strano-Rossi et al 2010). MDPV: methylenedioxypyrovalerone

A metabolic study of BZP in Wistar rats and an analysis of urine samples from human users indicated that only a small proportion of BZP is converted into metabolites (Staack, Fritschi et al. 2002; Staack and Maurer 2005) (Figure 9). Studies on human volunteers showed that after a single oral dose of 200 mg BZP hydrochloride, only 12.25% was excreted in urine, which may be explained by low bioavailability, other excretion routes or strong binding to tissue or protein (Antia, Lee et al. 2009).

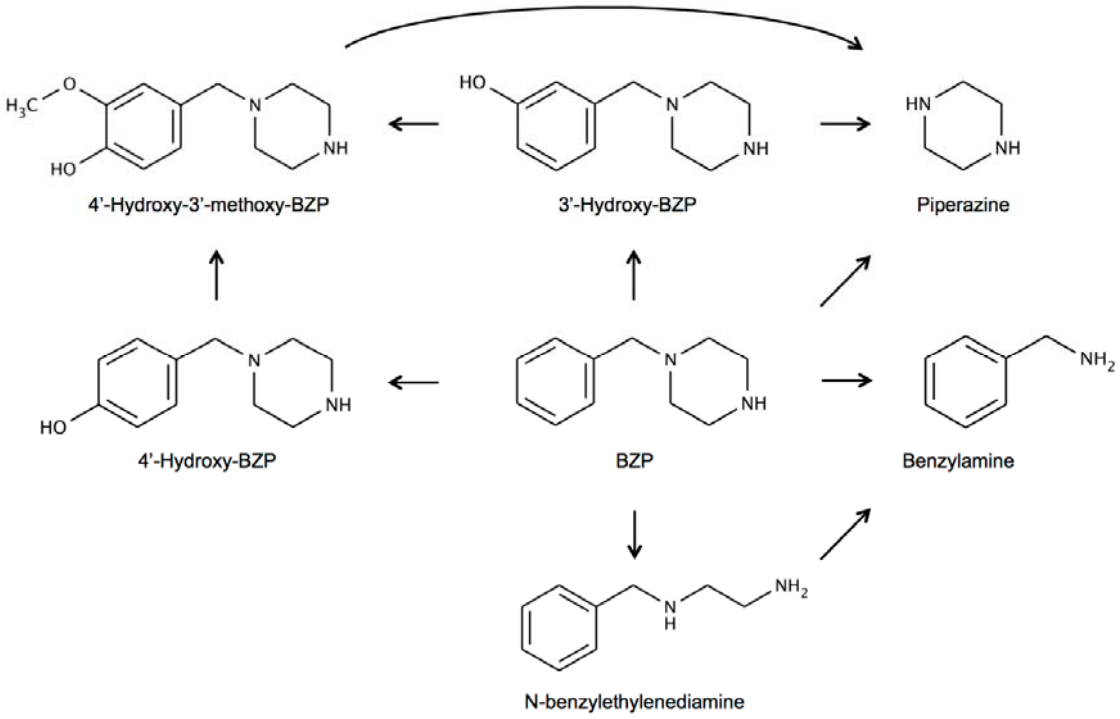


Figure 9. Proposed metabolic pathways of BZP in humans (derived from Staack et al 2002). BZP: benzylpiperazine

Unlike BZP, TFMPP is intensively metabolised via multiple pathways such as aromatic hydroxylation and N-dealkylation, resulting in a variety of metabolites (Staack, Fritschi et al. 2003; Staack and Maurer 2005; Tsutsumi, Katagi et al. 2005) (Figure 10). The percentage of the administrated TFMPP dose found in urine was low as less than 1% (Antia, Tingle et al. 2010).

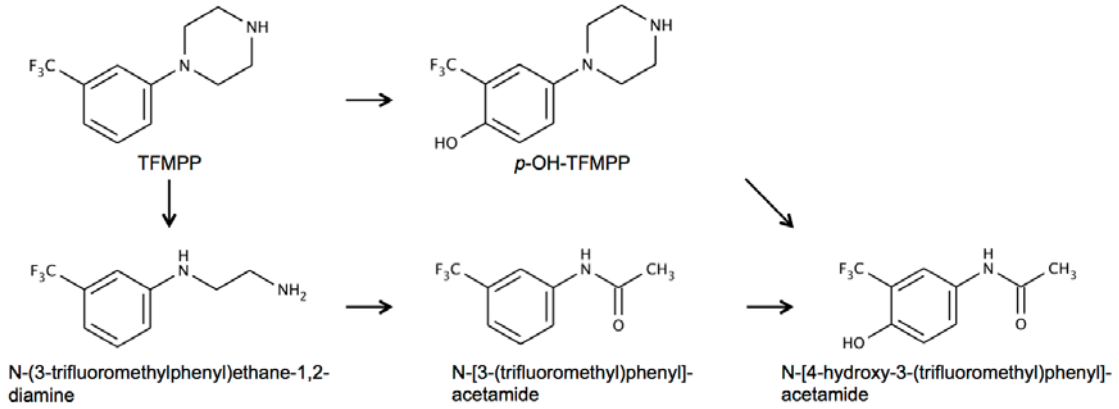


Figure 10. Proposed metabolic pathway of TFMPP in humans (derived from Tsutsumi et al 2005). TFMPP, 3-trifluoromethylphenylpiperazine; p-OH-TFMPP, 4-hydroxy-3-trifluoromethylphenylpiperazine

5.2.5. Summary

The metabolic pathways of drugs and the excretion ratio of each metabolite are essential information for analytical target determination and back-calculation in wastewater epidemiology studies. For the popular stimulants cocaine, methamphetamine and MDMA, such data from healthy volunteers are readily available. However, care needs be taken when applying these data to wastewater studies, since these data may not represent the user population. Administration routes may have a significant impact on plasma drug levels, peak times and excretion patterns, which need to be taken into consideration in the back-calculation process (Khan and Nicell 2011; Khan and Nicell 2012). Factors such as dose (de la Torre, Farre et al. 2004), co-administration of alcohol (Hoyumpa 1984; Shimosato 1988) (which is common) and gender (Moody, Fang et al. 2011) may also cause variations in drug metabolism and pharmacokinetics.

For the novel synthetic stimulants, excretion ratios of the metabolites from humans are largely unknown due to the lack of clinical studies. This limitation obstructs the

back-calculation of the consumption of these drugs in the population from excretion data obtained via wastewater analysis. However, animal tests and analysis of human specimens suggested that the original forms of these drugs exist in the urine (although the percentage is less than 1% for TFMPP) (Staack, Fritschi et al. 2002; Staack, Paul et al. 2004; Kamata, Shima et al. 2006; Antia, Lee et al. 2009; Meyer, Du et al. 2010; Pedersen, Reitzel et al. 2012), hence the parent drugs can be used as analytical targets for geographical or temporal comparisons in wastewater epidemiology studies.

5.3. Methods

Currently, the most widely applied approach for wastewater epidemiology is the one described by Zuccato et al (2008). Firstly, untreated wastewater samples (preferably 24-hour composite samples collected by an autosampler in a flow-proportional manner, but grab samples are also used) are collected from a WWTP, and stored there for periods ranging from hours to days before being transferred to the analytical laboratory. Storage may also occur after arriving at the laboratory and prior to instrumental analysis. A variety of extraction methods (mostly off-line or on-line solid-phase extraction, SPE), separation techniques (HPLC, UPLC or GC) and mass spectrometers equipped with different ion sources are used to prepare and analyse the samples. Internal standards are often spiked into the samples prior to preparation and used for quantification. Following the determination of analytical target concentrations, the excreted amount of analyte is calculated by multiplying the measured concentration with the wastewater volume entering the WWTP. Then the consumption of the drug of interest is back-calculated considering

the excretion ratio of the analytical target and mass difference between the parent drug and metabolite if a metabolite instead of the parent drug is measured. Finally, the prevalence of the illicit drug is estimated by dividing the total consumption by the local census population, and expressed as mg *per* day *per* 1000 people in the contributing area. This value can be further transformed to dose data if a standard dose of this drug is known or assumed. Figure 11 summarises the procedures of the wastewater epidemiology method, including sample collection, storage, preparation, analysis and data conversion.

Lai et al (2011) assessed the uncertainly of five parameters in the whole process, namely sampling, analyses of chemicals, measurements of wastewater flow, determination of excretion rates and estimation of population. The analysis suggested that with the best practice currently available, the uncertainty values (relative standard deviation, RSD) of estimated drug consumption in the contributing area are 21% for cocaine and methamphetamine, and 22% for MDMA. Considering the structure similarity of the novel synthetic stimulants to these popular stimulants, the uncertainty values of the novel synthetic stimulants are assumed to be in the same range.

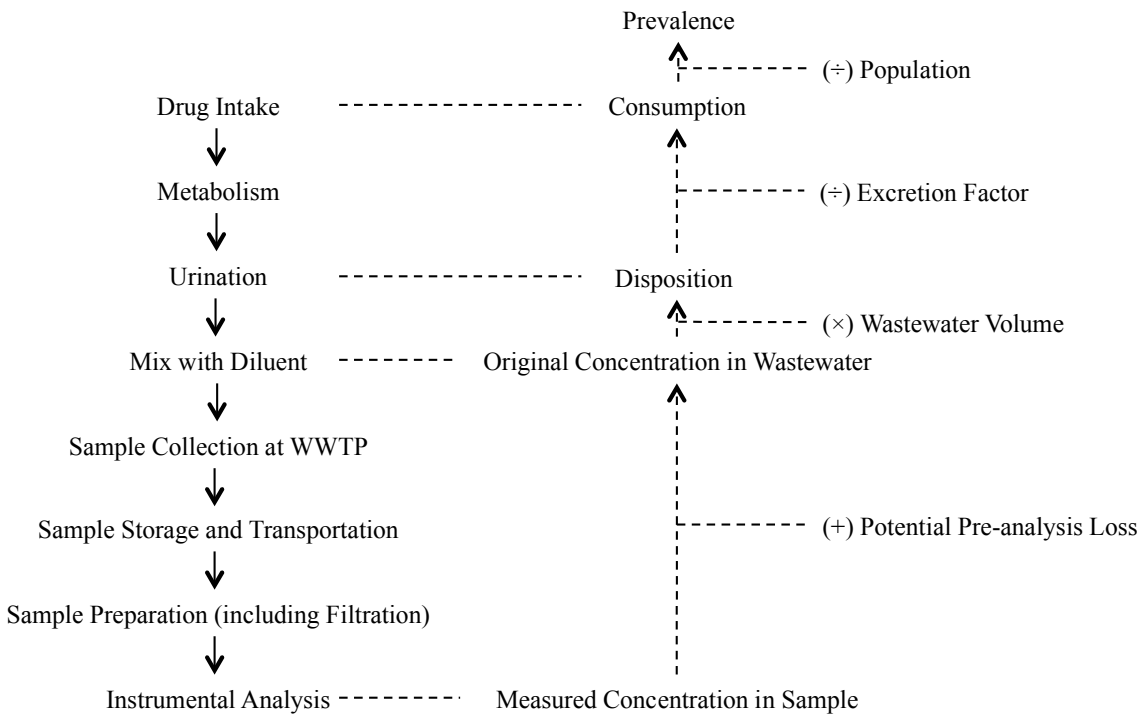


Figure 11. Current procedures of wastewater epidemiology studies

5.4. Applications

Because of the objectiveness, timeliness, and cost efficiency of the wastewater epidemiology method, it has been applied in several countries for the estimation of community use of illicit drugs. Table 1 reviews all such studies prior to the commencement of this project, with some differences in sampling, analysis and data presentation.

Table 1. Studies published by 2009 on the analyses of illicit drugs and metabolites in wastewater and surface water.

Analyte	Sampling type	Sampling site and period	Sample storage	Sample preparation	Analytical method	Reference
2 × ATS 1 × antidepressant	Time-weighted POCIS	1 WWTP each in Nevada, Utah, and South Carolina (USA); 28–30 days in summer 2002 (Nevada resampled in winter 2003)	Not reported	POCIS Extraction	HPLC–ITMS	(Jones-Lepp, Alvarez et al. 2004)
2 × CRS	Time-dependent composite samples (20 min intervals for 24 h at WWTPs; 30 min intervals for 2.5 h from SW)	4 WWTPs and 1 river (Italy), sampling period not reported	At 4 °C for max. 3 days	SPE (Oasis-MCX)	HPLC-ITMS/TQMS	(Zuccato, Chiabrando et al. 2005)
5 × CRS 5 × ATS 5 × opioids 1 × cannabinoid	Time-dependent (20 min intervals) 24-h composite samples	1 WWTP in Milan-Nosedo (Italy) and 1 WWTP in Lugano (Switzerland); in Feb and Mar 2006	In dark glass bottle at 4 °C for max. 3 days	SPE (Oasis-MCX)	HPLC- TQMS	(Castiglioni, Zuccato et al. 2006)
1 × CRS 7 × opioids 6 × benzodiazepines 4 × antidepressants 2 × others	Grab samples	12 WWTPs, 4 rivers and 7 creeks in Germany; Mar and Nov 2005	In glass bottles at 4 °C for max. 1 day	SPE (Oasis HLB)	HPLC- TQMS	(Hummel, Loffler et al. 2006)

Analyte	Sampling type	Sampling site and period	Sample storage	Sample preparation	Analytical method	Reference
9 × opioids 2 × cannabinoids	24-h composite samples at WWTPs, grab samples from SW	5 WWTPs in Catalonia (NE Spain) from Mar to May 2007. 1 river (Spain) (sampling period not reported)	In dark bottles at 4 °C for max. 3 days	SPE (Oasis HLB)	UPLC- TQMS	(Boleda, Galceran et al. 2007)
3 × CRS 1 × ATS 3 × opioids 1 × lysergic 1 × antidepressant 2 × benzodiazepines	24-h composite samples at all except 1 WWTP, grab samples from SW	5 WWTPs and receiving river in Dublin (Ireland) during the week beginning Nov 20th, 2006	For max. 24 h, condition not reported.	SPE (Strata-XC)	HPLC- ITMS	(Bones, Thomas et al. 2007)
2 × CRS 5 × ATS 1 × opioid 2 × anaesthetics 1 × lysergic 4 × others	24-h composite samples at WWTPs, grab samples from SW	16 WWTPs in Catalonia (NE Spain) from Apr to Sep 2006. 1 river (Spain) in Sep 2006	In glass bottles in the dark at < 4 °C for max. 1 day	SPE (Oasis HLB)	UPLC- TQMS	(Huerta-Fontela, Galceran et al. 2007)
3 × CRS 4 × ATS 3 × opioids 3 × lysergic 3 × cannabinoids	24-h composite samples	1 WWTP in Barcelona during the first week of Jul 2007, 1 WWTP each in Valencia, Benicasim and Gandia on 26th of Jul 2007 (Spain)	Filtered and stored in glass bottles in the dark at –20 °C.	SPE (Oasis HLB for cannabinoids, PLRP-s for the others)	HPLC- TQMS	(Postigo, Lopez de Alda et al. 2008)

Analyte	Sampling type	Sampling site and period	Sample storage	Sample preparation	Analytical method	Reference
4 × CRS 7 × ATS 3 × opioids 3 × anaesthetics 2 × lysergics 2 × benzodiazepines 3 × others	24-h flow normalised composite samples	7 WWTPs (USA), sampling period not reported	In polypropylene bottles at 4 °C, shipped frozen and stored at –20 °C for max. 3 weeks.	LVI	HPLC- TQMS	(Chiaia, Banta-Green et al. 2008)
3 × CRS	24-h composite samples at WWTPs, grab samples from SW	5 WWTPs in Flanders and 3 rivers (Belgium), sampling period not reported	pH-adjusted to 2 and stored in glass bottles at –20 °C	SPE (Oasis HLB, other cartridges tested)	HPLC(HILIC)-ITMS	(Gheorghe, van Nuijs et al. 2008)
2 × CRS 5 × ATS 1 × opioid 1 × lysergic 2 × anaesthetics 4 × others	Grab samples	42 WWTPs (NE Spain), once from each between Apr 2006 and Apr 2007	Stored in the dark at < 4 °C for max. 1 day	SPE (Oasis HLB)	UPLC-TQMS	(Huerta-Fontela, Galceran et al. 2008)
2 × CRS 1 × ATS 2 × opioids 1 × antidepressant 48 × others	Grab samples	1 WWTP and 1 river in Wales (UK), sampling period not reported	In silanised amber bottles, adjusted to pH 2 and stored at 4 °C	SPE (Oasis MCX, other cartridges tested)	UPLC-TQMS	(Kasprzyk-Hordern, Dinsdale et al. 2008a)

Analyte	Sampling type	Sampling site and period	Sample storage	Sample preparation	Analytical method	Reference
1 × CRS 1 × ATS 1 × opioid 53 × others	Grab samples	2 rivers (UK) at least once a month over a period of 10 months (Nov 2006–Aug 2007)	In silanised amber bottles, acidified to pH 2.0 and filtered	SPE (Oasis MCX)	UPLC-TQMS	(Kasprzyk-Hordern, Dinsdale et al. 2008b)
3 × CRS 4 × ATS 6 × opioids 1× cannabinoid	Time-dependent (20 min intervals for 2 h) composite samples	3 rivers (2 in Italy, 1 in UK) and 3 lakes (Italy). The UK river sample was collected in Oct 2005 and all others in 2006.	Not reported	SPE (Oasis MCX)	HPLC- TQMS	(Zuccato, Castiglioni et al. 2008)
2 × CRS 3 × ATS 2 × opioids 1 × cannabinoid	Time-dependent composite samples (20 min intervals for 24 h)	1 WWTP in Milan (Italy) in Nov 2005, Feb 2006 and Mar 2006; 1 WWTP in Lugano (Switzerland) in Mar 2006; 2 WWTPs in London (UK) in Oct 2005	Not reported	SPE (Oasis MCX)	HPLC-TQMS	(Zuccato, Chiabrando et al. 2008)
1 × CRS 2 × ATS	24-h composite samples	96 WWTPs in Oregon (USA), on 4 Mar 2008	Transported within 3 days of collection and frozen at –20 °C until analysis	LVI	HPLC- TQMS	(Banta-Green, Field et al. 2009)
1 × anaesthetic 2 × ATS 16 × others	POCIS	5 rivers in Nebraska (USA); Aug and Oct 2006	Not reported	POCIS Extraction	HPLC-TQMS	(Bartelt-Hunt, Snow et al. 2009)

Analyte	Sampling type	Sampling site and period	Sample storage	Sample preparation	Analytical method	Reference
5 × CRS 5 × ATS 1 × cannabinoid	24-h composite samples	1 WWTP (Spain); during the third week of Jun and the third week of Jul 2008.	In polyethylene bottles and stored in the dark at −20 °C	SPE (Oasis MCX)	UPLC- TQMS	(Bijlsma, Sancho et al. 2009)
9 × opioids 2 × cannabinoids	24-h composite samples (1 h intervals) at WWTPs, grab samples from SW	15 WWTPs from March to May 2007; 1 river in spring and autumn 2007	In dark bottles at –4 °C for max.3 days	SPE (Oasis HLB)	UPLC- TQMS	(Boleda, Galceran et al. 2009)
5× ATS	Grab samples	4 WWTPs (NW Spain); during Jun 2009	In amber glass bottles and stored in the dark at 4 °C	SPE (SupelMIP-Amphetamine)	HPLC-TQMS	(Gonzalez-Marino, Quintana et al. 2009)
2 × CRS 1 × ATS 17 × others	24-h composite samples /grab samples	2 WWTPs in South Wales (UK), Apr 2007–Aug 2007	In silanised bottles, acidified to pH 2.0 and filtered	SPE (Oasis MCX)	UPLC-TQMS	(Kasprzyk-Hordern, Dinsdale et al. 2009a)
2 × CRS 1 × ATS 2 × opioids 1 × antidepressant 47 × others	24-h composite samples at WWTPs, grab samples from SW	2 WWTPs and 2 rivers (UK), Apr 2007–Aug 2007	In silanised bottles, acidified to pH 2.0 and filtered	SPE (Oasis MCX)	UPLC-TQMS	(Kasprzyk-Hordern, Dinsdale et al. 2009b)
2 × ATS 4 × others	Grab samples	1 WWTP and 1 creek (USA); on 5 days from Sep 2006 to Sep 2007	In polyethylene bottles placed in a cooler, and stored at –20 °C	SPE (Oasis HLB)	HPLC–ITMS	(Loganathan, Phillips et al. 2009)

Analyte	Sampling type	Sampling site and period	Sample storage	Sample preparation	Analytical method	Reference
2 × CRS 1 × opioid	Grab samples after sedimentation (representing 24 h)	2 WWTPs in Florence (Italy); every first Monday of the month from Jul 2006 to Jun 2007	At 4 °C for max. 3 days	SPE (Bond Elut Certify LRC)	GC-ITMS	(Mari, Politi et al. 2009)
3 × CRS 3 × ATS 3 × opioids	Volume-proportional 24-h composite samples	11 WWTPs (Belgium), sampling period not reported	In glass bottles, pH-adjusted to 2 and stored at –20 °C	SPE (Oasis MCX)	HPLC (HILIC)-TQMS	(van Nuijs, Tarcomnicu et al. 2009)
3 × CRS	24-hour flow-dependent composite samples	41 WWTPs (Belgium) during summer of 2007 and the winter of 2007–08	In glass bottles stored at pH 2 and –20 °C	SPE (Oasis HLB)	HPLC(HILIC)-ITMS	(van Nuijs, Pecceu et al. 2009a)
3 × CRS	24-hour flow-dependent composite samples at WWTPs, grab samples from SW	30 WWTPs in summer–autumn of 2007 and winter of 2007–2008, 6 rivers and brooks in summer of 2007 and winter of 2007–2008	In glass bottles stored at pH 2 and –20 °C in the dark	SPE (Oasis HLB)	HPLC(HILIC)-ITMS	(van Nuijs, Pecceu et al. 2009c)
3 × CRS	24-hour flow-dependent composite samples at WWTPs, grab samples from SW	37 WWTPs and 28 rivers and brooks from Jun 2007 to Feb 2008	In glass bottles stored at pH 2 and –20 °C	SPE (Oasis HLB)	HPLC(HILIC)-ITMS	(van Nuijs, Pecceu et al. 2009b)

ATS: Amphetamine-type stimulant; CRS: cocaine-related substance; FT: Fourier transforms; GC: gas chromatography; HPLC: high performance liquid chromatography; HILIC: hydrophilic interaction; ITMS: ion trap mass spectrometry; LVI: large-volume injection; POCIS: polar organic chemical integrative sample/sampler; RPLC: reverse-phase liquid chromatograph; SPE: solid-phase extraction; SW: surface water; TQMS: triple quadruple mass spectrometer; UPLC: ultra performance liquid chromatograph; WWTP: wastewater treatment plant

5.5. Advantages and current issues

It is apparent that the method of wastewater epidemiology is of great value to researchers and authorities. Firstly, it avoids the bias in interviews and surveys, thereby providing more reliable results. Besides, the major procedures of this approach are simply taking some samples from local sewage pipes and analysing them. This universality allows development of standardised approaches for international comparisons. Moreover, it only takes a couple of days from sample collection to result submission, which allows near real-time monitoring of community drug use while other approaches do not (Daughton 2001). Besides, this monitoring method is capable of assessing marginalised groups and remote areas, which is difficult for interview-based surveys.

Although the advantages are obvious, this wastewater epidemiology approach is not yet perfect. Some technical issues and uncertainties are involved in each step of the analysis and calculation, as shown in Figure 11.

In the concentration measurements, uncertainties or errors may occur since a number of steps are involved in the sample treatment and analysis, and each of them can generate issues. Baker and Kasprzyk-Hordern (2011a) evaluated factors in the sample preparation (filtration, SPE, evaporation temperature and silanising glassware) and suggested that all of these factors are vital in developing efficient and reliable extraction techniques.

Furthermore, the measured concentrations of analytes may not equate to the original concentrations of excreted analytes, since decomposition may occur between excretion and analysis (van Nuijs, Castiglioni et al. 2011). Information on analyte stability in wastewater

is vital to evaluate if there is significant decomposition between drug excretion and sample collection. Also, if the analyte binds to particulate matter in wastewater or the filter paper, it will be removed in the essential filtration step in sample preparation.

The population data used in the back-calculation are normally based on infrequent census data, which do not reflect the actual contributing population of the analysed wastewater sample.

Daily wastewater volume measured at a WWTP is also essential. This parameter is determined by a flow meter installed in the wastewater pipeline. Lai et al (2011) reported the flow measurement to be a major uncertainty for estimating drug excretion, and a conscientious calibration of flow meters is required to reduce it.

As previously discussed in Section 5.2 of this chapter, the excretion factors of illicit drugs are largely unknown, with limited information from *in vitro* experiments, animal tests or small scale clinical studies. This lack of information is an obstacle which prevents accurate estimation of population drug use via wastewater analysis.

Apart from these technical issues, if authorities use this approach to monitor a closed water system such as a sewage pipe from a particular building or a local neighbourhood, ethical and privacy concerns will arise. Residents may think that they are being spied on by the government through their lavatories (Frost, Griffiths et al. 2008).

Most importantly, wastewater analysis does not reveal the contributor of the sample, hence is unable to link the prevalence of drugs to the users' profiles, or suggest the prevalence in

subgroups (e.g. young people or male/female groups). In contrast, this information is readily available with traditional surveys.

To summarise, wastewater analysis is a promising method that provides objective information on population drug use in a cost effective and time efficient way. However, some technical issues need to be resolved before this method can be considered reliable. Besides, this method focuses on the prevalence of drug use within the whole population and is unable to narrow down to subgroups, due to the fact that this method cannot provide user information. Hence, after validation and further development, wastewater analysis can serve as an important complementary data source to large scale surveys. This further suggests that the application of wastewater analysis is of great value in obtaining information on population drug use, which is unavailable with traditional methods. It should be also noted that while the World Drug Report suggested that Australia shows higher use of ATS than other countries (UNODC 2009), wastewater analysis had not been applied in Australia at the commencement of this project.

6. Research aims

The initial aims of this project were to validate one critical part of the wastewater analysis method, namely the pre-analysis loss of analytes, and then apply this approach in South Australia to gain some information on illicit stimulant use in the population, which is not obtainable via traditional methods. With the progress of this project and some new trends emerging in the population (e.g. a suggested MDMA decline with increased use in novel

synthetic stimulants), the aims were later broadened to the monitoring of illicit stimulants in the population, for both "traditional" illicit stimulants and novel "legal highs".

6.1. Method validation: evaluation of pre-analysis loss

Potential pre-analysis loss of analytes may occur because of stability and binding issues. However, previous studies on stability (Castiglioni, Zuccato et al. 2006; Chiaia, Banta-Green et al. 2008; Gheorghe, van Nuijs et al. 2008; Bisceglia 2010; Gonzalez-Marino, Quintana et al. 2010; Baker and Kasprzyk-Hordern 2011a; Castiglioni, Bagnati et al. 2011) did not systematically compare the storage conditions evaluated, hence which condition is the most appropriate for wastewater epidemiology studies is unclear. Previous binding assessment did not exclude the possibility of irreversible binding of analytes (Metcalfe, Tindale et al. 2010).

This study aimed to further validate the wastewater analysis method by evaluating if there is significant analyte loss between human excretion and instrumental analysis; and if so, whether the loss can be avoided or compensated. The investigated substances and metabolites included: cotinine, cocaine and its metabolite, phenethylamines and opioids. The storage conditions compared included: untreated, storage at 4 °C, –20 °C, low pH (acidification), preservative, and pre-filtration. Binding tests were conducted by way of evaluating analyte recoveries extracted via liquid-liquid extraction (LLE) before and after filtration.

6.2. Application in South Australia

Following validation, the method of wastewater analysis was applied to the State of South Australia, Australia in the hope of obtaining information on illicit stimulant use in the population. The aim was to compare illicit stimulant use in metropolitan areas and regional areas, between weekdays and weekends, and between Australian and European countries. Comparison of data collected via wastewater analysis with those via surveys can also serve to evaluate the two monitoring techniques.

6.3. Monitoring of MDMA use

During late 2009 and early 2010, a decline in MDMA purity in tablets has been suggested by mass media and drug reporting systems (UNODC 2010). However, whether this was a global phenomenon was unsure, and whether this decline in MDMA supply was associated with increases in the use of other major illicit stimulants was unknown. The aim of this study was to provide timely and objective data to answer these two questions using the validated method of wastewater analysis.

6.4. Novel synthetic stimulants in wastewater

Previous studies have confirmed that there was a decline in MDMA use in the population from 2009 to 2010 in Adelaide, Australia, which was not fully compensated for by cocaine and methamphetamine (Chen, Kostakis et al. 2011). Meanwhile, the reporting system noticed an increased prevalence of novel synthetic stimulants (UNODC 2010). Hence, the decline in MDMA in 2009/2010 may have been attributable to a corresponding increase in

novel synthetic stimulants. The aim of the study was to investigate if such a correlation existed, or whether the two phenomena were coincidental. Which hypothesis is correct is crucial information for authorities in demonstrating whether controlling the use of one drug will promote the use of another. The studied novel synthetic stimulants include synthetic cathinones methcathinone, mephedrone, methylone and MDPV, as well as synthetic piperazines BZP and TFMPP.

CHAPTER 2 "Evaluation of pre-analysis loss of dependent drugs in wastewater: stability and binding assessments" (Publication 1)

Wastewater analysis has shown the potential to provide supplementary information on population drug use, and has been applied in a number of countries. However, some critical validation work is required before the method can be considered reliable.

One major concern was whether the measured levels of drugs or metabolites in the samples reflected the original amounts of drug excretion. Changes in the concentrations of drugs and metabolites in the wastewater or prepared samples during the period between urinary excretion and instrumental analysis are possible. Decomposition and formation of these analytes may occur in a number of circumstances and periods, including the lag-time between urine excretion and wastewater sample collection, the period between sample collection and preparation, and the delay prior to instrumental analysis. Analyte loss may also occur during the filtration step in sample preparation, since wastewater contains a large amount of particulate matter that need to be removed before further treatment. Therefore, determining whether analytes bind to these particulate matter or filter paper is also essential.

The result of this study showed that most studied drugs or metabolites are stable in wastewater, and storing wastewater samples at 4 °C is sufficient to stabilise the analytes in most circumstances. There was no noticeable loss of analytes after filtration or in the sample extract prior to instrumental analysis. Because of the stability issue, COC was

confirmed as an unsuitable analytical target for the estimation of population cocaine use, and its metabolite benzoylecgonine should be targeted instead. For heroin use monitoring, it is recommended to preserve wastewater samples with 2 g/L sodium metabisulphite ($Na_2S_2O_5$) and target the exclusive heroin metabolite 6-monoacetylmorphine. These observations have been incorporated into the subsequent studies to improve the reliability of the results.

Chen, C., Kostakis, C., Irvine, R.J., Felgate, P.D. & White, J.M. (2012) Evaluation of pre-analysis loss of dependent drugs in wastewater: stability and binding assessments.
Drug Testing and Analysis, v. 5(8), pp. 716-721

NOTE:
This publication is included on pages 58-72 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.1002/dta.1428

CHAPTER 3 "Population drug use in Australia: a wastewater analysis" (Publication 2)

At the commencement of this PhD project, a number of wastewater epidemiology studies had been conducted in some European countries and the United States (Table 1), which presented valuable findings. However, no such studies were carried out in Australia. Considering Australia is a geographically isolated continent with higher reported use of ATS than other countries in the world (UNODC 2009), it was worthwhile examining if the method of wastewater epidemiology was applicable in Australia. It was also considered important to compare the wastewater data obtained in Australia with those from other countries, and with traditional survey data collected in Australia.

This first application of wastewater epidemiology method in Australia showed that among all the three illicit stimulants, methamphetamine is the most popular in South Australia; weekend consumption of all of the three stimulants is higher than weekday consumption, with MDMA displaying the largest difference between weekend and weekday use. Metropolitan areas show higher prevalence of methamphetamine and cocaine use, whereas regional areas have higher MDMA use. Compared with London and Milan, Adelaide (the capital of South Australia) showed 10–40 times higher MDMA and methamphetamine use, yet only about 1/30 of cocaine consumption. These international differences had been reported but largely underestimated in previous World Drug Reports (UNODC 2008; UNODC 2009).

This application also confirmed the usefulness of this epidemiological approach, and hence stimulated the following studies aiming to obtain more information on stimulant use in the population.

Irvine, R.J., Kostakis, C., Felgate, P.D., Jaehane, E.J., Chen, C. & White, J.M. (2011) Population drug use in Australia: a wastewater analysis.
Forensic Science International, v. 210(1-3), pp. 69-73

NOTE:
This publication is included on pages 75-79 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.1016/j.forsciint.2011.01.037

CHAPTER 4 "Marked decline in 3,4-methylenedioxymethamphetamine (MDMA) based on wastewater analysis" (Publication 3)

From late 2009 to early 2010, a decrease in the availability of MDMA was reported in the media, particularly in Europe (EMCDDA 2010b), but direct and quantitative measurement was unavailable to verify this trend. It was also uncertain whether there was a parallel decrease in MDMA consumption in Australia, and if there was related change in use of other illicit stimulants, as had been seen previously (UNODC 2010). The aim of this study was to establish if a decrease in MDMA consumption occurred in Adelaide from 2009 to 2010 using the method of wastewater analysis. Use of the related stimulants methamphetamine and cocaine were also determined in both years for comparison.

Results clearly showed that MDMA consumption dropped from a level highest in the world to almost zero over the period of measurement. A slight increase in methamphetamine consumption was found but not sufficient to fully compensate for the decline in MDMA.

The reason for the MDMA decline was uncertain. International control efforts may have played a role, but some reports (EMCDDA 2010a; EMCDDA 2010b; Health Research Board 2010; UNODC 2010) suggested an emergence of synthetic MDMA alternatives during the MDMA decline. This issue forms the basis for the studies described in Chapter 5.

Marked Decline in 3,4-Methylenedioxymethamphetamine (MDMA) Based on Wastewater Analysis

CHANG CHEN, M.MED.,† CHRIS KOSTAKIS, M.APP.SC.,† PETER HARPAS, B.APP.SC.,† PETER D. FELGATE, B.SC. (HONS.),† RODNEY J. IRVINE, PH.D., AND JASON M. WHITE, PH.D.†

Discipline of Pharmacology, University of Adelaide, Adelaide, SA 5005, Australia

ABSTRACT. Objective: Recent reports in Europe suggest a decline in 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) use, but quantifiable and objective measurement is unavailable. The global extent of changes in MDMA and related stimulant use is also unclear. This study aims to quantify changes in MDMA use in Australia and determine whether these changes have been accompanied by differing amounts of other stimulant use. **Method:** We acquired information on recent use of MDMA and related illicit stimulants in Australia using the method of wastewater analysis. Untreated wastewater samples collected from three metropolitan treatment plants in Adelaide from May to July 2009 and the same months in 2010 were analyzed. Concentrations of MDMA, methamphetamine, and benzoylecgonine (a metabolite of cocaine) were determined using solid phase extraction–liquid chromatography–tandem mass spectrometry. Weekly consumed doses of MDMA, methamphetamine, and cocaine per 1,000 people were estimated. **Results:** From 2009 to 2010, weekly consumption of MDMA decreased from mean of 4.52 (*SEM* = 0.74) doses/week per 1,000 people to 0.08 (0.01) doses/week per 1,000 people ($p < .001$); weekly consumption of methamphetamine increased from a mean of 48.35 (6.13) doses/week per 1,000 people to 68.13 (5.33) doses/week per 1,000 people ($p < .05$); and weekly consumed doses of cocaine did not significantly change. Local roadside saliva testing data also showed that the MDMA-positive test rate decreased from 0.30% to 0.05% and the methamphetamine-positive test rate increased from 1.43% to 1.52% during the past 2 years. **Conclusions:** This study shows a 50-fold decrease in consumed doses of MDMA with a rise in methamphetamine use in Australia over a 1-year period. (*J. Stud. Alcohol Drugs, 72,* 737–740, 2011)

RECENTLY, A DECREASE IN THE AVAILABILITY of 3,4-methylenedioxymethamphetamine (MDMA) with decreased MDMA purity in Ecstasy tablets has been reported in the media and noticed by some organizations monitoring drug use (European Monitoring Centre for Drugs and Drug Addiction, 2010b; United Nations Office on Drugs and Crime, 2010). However, these reports were based on drug seizures and small-scale surveys, which depended heavily on occasional cases and self-reporting, with the limitations associated with that method of measurement. Also, these reports were focused on Europe, and it is unclear whether this trend is a global one.

In 2005, a novel monitoring approach for illicit drugs based on wastewater analysis was developed (Zuccato et al., 2005). This method determines the concentration of drug residues in wastewaters of specific localities and back-calculates the prevalence of drugs in the related population. The approach has been recognized as reliable and rapid, and, as a result, it was soon applied in several countries to assess local drug use (van Nuijs et al., 2010).

Australia is an ideal sampling country to investigate the change in the global use pattern of MDMA for two reasons. First, Australian MDMA prevalence has traditionally been recognized as the highest in the world (United Nations Office on Drugs and Crime, 2010). Second, Australia has two systems, the Illicit Drug Reporting System and the Ecstasy and Related Drugs Reporting System, which together monitor national drug use routinely via surveys. This allows comparisons with drug use data collected from other sources. Currently, the Ecstasy and Related Drugs Reporting System surveys are showing a decline in purity and availability in MDMA reported by users (Sindicich and Burns, 2010).

We hypothesized that if the MDMA decline is a global one, wastewater analysis in Australia could provide objective and quantitative information to verify that fact. It is also important to determine concurrent use of methamphetamine and cocaine using this method to establish whether there was any "substitution" of either of these stimulants for MDMA. Hence, in this study we analyzed wastewater samples collected in Adelaide, Australia, in 2009 and 2010 and compared the estimated weekly consumed doses of MDMA, methamphetamine, and cocaine across the years. We also obtained results from random roadside saliva testing carried out by the South Australian police to assess whether the findings derived from the two sources were consistent.

Method

Sample collection

Wastewater samples were collected from three independent metropolitan treatment plants, which together serve 1.3 million residents in Adelaide. Twenty-four–hour (8:00 A.M.–8:00 A.M.) untreated wastewater samples were collected

Received: March 30, 2011. Revision: May 10, 2011.

†Correspondence may be sent to Chang Chen at the above address or via email at: chang.chen@adelaide.edu.au. Chris Kostakis, Peter Harpas, and Peter D. Felgate are with Forensic Science South Australia, Adelaide, Australia. Jason M. White is with School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia.

in a flow-dependent manner using autosamplers after initial screens in which large solids were removed. Aliquots (1.2 L) of the composite samples were then transferred into plastic bottles and stored at -20 °C until analysis.

Samples were collected at the same time of year (from May to July) in 2009 and 2010. In 2009, samples were collected from each of the three plants once every 6 days from May to July. In 2010, samples were collected every day for 3 weeks in May from Plant 1, for 2 weeks in June from Plant 2, and for 3 weeks in July from Plant 3. A total of 42 samples were analyzed in 2009 and 56 were analyzed in 2010, which eventually provided 6 batches of weekly data for 2009 and 8 batches of weekly data for 2010. No public holidays or large entertainment events took place near the sampling days.

Sample analysis

Analysis was based on the method reported previously (Irvine et al., 2011). Generally, thawed and mixed samples were filtered under vacuum using glass microfiber filters (GF/A 1.6 µm; Whatman Ltd., Kent, UK). Deuterated internal standards of MDMA, methamphetamine, and benzoylecgonine (a metabolite of cocaine) were added to 300 mL of duplicate samples to give resultant concentrations of 33.3, 33.3, and 166.7 ng/L, respectively. Acetic acid (2.5%) was added to lower the pH of the samples to 4.5–5. The acidified samples were then loaded onto preconditioned mixed-mode solid-phase extraction cartridges (XRDAH506; UCT Inc., Bristol, PA). The cartridges were successively washed with 6 mL of pH 5.7 acetate buffer, 2 mL of 0.1 M acetic acid, and 6 mL of methanol. Analytes were eluted with a mixture of 96% dichloromethane:i-propanol (80:20) / 4% concentrated ammonia and evaporated to dryness. The residue was reconstituted into 20 µL of methanol and then mixed with 180 µL of 0.1% formic acid. A set of diluted extracts were prepared by transferring 40 µL of the original extract to new vials and diluting each with 160 µL of 0.1% formic acid. Both sets were analyzed by liquid chromatography–tandem mass spectrometry.

Chromatographic separation was carried out using an Agilent 1200 series liquid chromatograph with a pentafluorophenylpropyl (PFP[2]) column (Luna 3 µm, 100 Å, 50 × 4.6 mm; Phenomenex Inc., Torrance, CA) connected to a PFP(2) guard column (SecurityGuard; 4 × 2.0 mm; Phenomenex Inc., Torrance, CA). Mass spectra were obtained using a 4000 Q-Trap (Applied Biosystems Ltd., Toronto, Canada) system equipped with an electrospray ionization source operated in positive mode via multiple-reaction monitoring.

Data analysis

MDMA and methamphetamine concentrations were determined in the diluted set by isotopic dilution using their corresponding deuterated analogues. Benzoylecgonine concentration was determined in the same way using the undiluted set. Estimation of drug consumption was based on the method recently reported by Zuccato et al. (2008). First, daily drug excretion was calculated by multiplying the concentration of drug residues in the wastewater samples by the daily influent wastewater volume. Then, daily consumption was estimated considering the excretion rate of each drug (MDMA and methamphetamine) or the metabolite (benzoylecgonine) from the human body. Daily drug consumption per 1,000 people was estimated using the local population data and then converted to doses/day per 1,000 people using "typical dose" data of Ecstasy (100 mg), methamphetamine tablets (30 mg), and cocaine (100 mg), respectively. Finally, weekly consumed drug doses were calculated by adding the daily doses over 7 days and expressed as doses/week per 1,000 of the population.

Data were analyzed by GraphPad Prism (GraphPad Software Inc., La Jolla, CA). Means and standard error of the mean of estimated weekly consumed doses were calculated for 2009 and 2010, respectively. Two-way analysis of variance and unpaired two-tailed *t* tests were applied as appropriate.

Results

Wastewater analysis

Figure 1 shows the estimated weekly consumed doses of MDMA, methamphetamine, and cocaine in 2009 and 2010. Weekly MDMA consumption decreased 98.3% during this period, whereas weekly use of methamphetamine increased 40.9%. Cocaine use did not significantly change during this period.

Roadside saliva testing

Roadside saliva testing, in which randomly selected drivers are tested for illicit drug use, was introduced in 2006 in South Australia as a means to reduce the number of fatalities and injuries that occur on the state's roads. From July 2008 to June 2009, 39,510 drivers were tested, of which 0.30% were positive for MDMA and 1.43% were positive for methamphetamine. In the following 12-month period, the number of drivers tested increased to 46,414, and the percentage of MDMA-positive drivers decreased markedly to 0.05% (an 83.3% decline), whereas the proportion of methamphetamine-positive drivers increased to 1.52%. No MDMA-positive tests were reported after March 2010, a period that includes the time when the wastewater samples were collected.

Discussion

The wastewater analysis data clearly showed a marked decline of MDMA use in Adelaide from 2009 to 2010 as well as increased use of methamphetamine during the same period. The roadside saliva testing data collected in the same

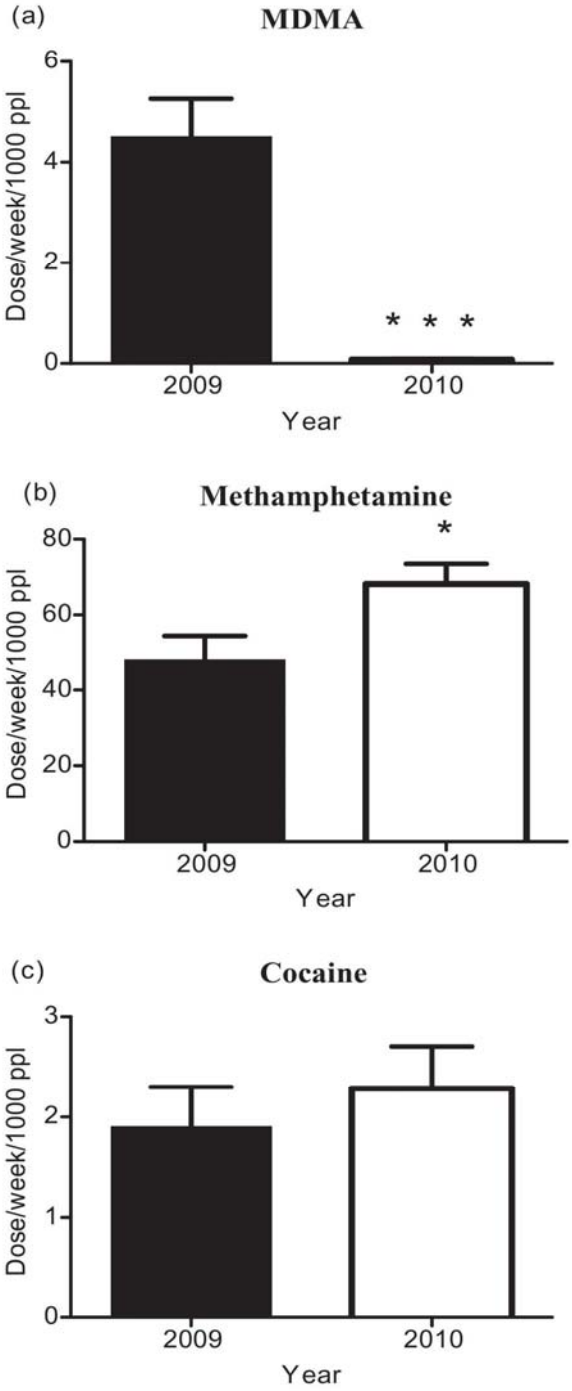


FIGURE 1. A comparison of estimated (a) 3,4-methylenedioxymethamphetamine (MDMA), (b) methamphetamine, and (c) cocaine weekly consumption in 2009 ($n = 6$) and 2010 ($n = 8$) in Adelaide expressed as doses/week per 1,000 people (ppl), mean ± *SEM*.
$^{*}p < .05$; $^{***}p < .001$, unpaired two-tailed t test.

period and city indicated the same trends. Survey data from the Ecstasy and Related Drugs Reporting System suggested that the percentage of regular Ecstasy users who reported the availability of Ecstasy as "difficult" and "very difficult" doubled from 12% to 26% from 2009 to 2010, and the proportion of respondents who reported low purity of Ecstasy increased from 24% to 56% (Sindicich and Burns, 2010). Thus, the data from wastewater analysis are consistent with survey data with regard to the direction of change in MDMA use, but the wastewater analysis shows a much greater magnitude of change than the surveys indicate. A recent report from the Illicit Drug Reporting System suggested stabilized cocaine use in Australia (Stafford and Burns, 2010), which also agreed with our data.

According to the National Campaign against Drug Abuse Household Surveys and National Drug Strategy Household Surveys in Australia from 1991 to 2007, the prevalence of MDMA use among the Australian population ages 14 and above increased in line with that of methamphetamine from 1995 to 1998. From 1998 to 2007, although the prevalence of MDMA continued growing, the prevalence of methamphetamine dropped gradually (United Nations Office on Drugs and Crime, 2010). Our data provided direct evidence of the first MDMA decline in Australia in the past 15 years. It is also worth mentioning that, when comparing this study with similar wastewater studies in Europe (Zuccato et al., 2008), it could be noted that the estimated MDMA consumption per day per capita in Adelaide in 2009 was approximately 10 times higher than that in Europe (Irvine et al., 2011). This agrees with the *World Drug Report 2010,* suggesting that Australian MDMA use was the highest in the world (4.2% annual use in the population ages 15–64 in 2007, and 3.6%–4% in 2008; United Nations Office on Drugs and Crime, 2010). Hence, the recent decline of MDMA use was from the highest level in the world to a level that is almost undetectable.

Based on the rationale of this method, we are not able to tell if the decreased MDMA consumption is a result of reduced consumption of Ecstasy tablets in the population or decreased purity of the Ecstasy tablets. However, the MDMA decline is noted to have corresponded with the growing prevalence of substitutes, such as mephedrone and meta-chlorophenylpiperazine (mCPP) in Europe (European Monitoring Centre for Drugs and Drug Addiction, 2010b; Health Research Board, 2010). In 2009, early-warning systems in Europe found that the proportion of Ecstasy tablets containing mCPP (or piperazines in general) increased markedly, possibly exceeding the Ecstasy tablets containing MDMA (European Monitoring Centre for Drugs and Drug Addiction, 2010a). Based on unpublished drug seizure data, this trend has also occurred in Australia, suggesting a possibility that the decline in MDMA use in Australia was compensated for by a rise in the use of derivatives. Because there is no evidence of a decline in interest in Ecstasy of "high quality," it

seems that a reduction in MDMA availability has driven the decline in its use.

It would be valuable if concentrations of the above-mentioned MDMA-like substances in the wastewater had been concurrently determined in this study. When carrying out similar studies in the future, it would be worth considering adding these MDMA alternatives to the list of analytical targets to have a full picture of stimulant use. However, this could be difficult because the types of MDMA alternatives in the drug market change frequently, and the number is rising (Elliott, 2011). To face the challenge, authorities, researchers, police, and customs agents need to collaborate efficiently and keep information on drug trends up to date (Elliott, 2011).

Similarly, it is not clear whether the increased use of methamphetamine is because of enlarged user population, higher daily doses consumed, or a combination of both. Using methamphetamine as a substitute of MDMA in Ecstasy tablets is also a possible explanation of the result of this study, although unpublished analysis of seized Ecstasy tablets suggests that this is not the case. It is possible that previous MDMA users changed to methamphetamine use with the decline in purity of Ecstasy tablets, and this accounts for the increase in methamphetamine use.

The sampling strategies were slightly different in 2009 and 2010 because of some practical reasons, but this would not have had a significant impact on the final conclusion of the study because the differences observed were very large.

Interestingly, although our data suggested that MDMA has almost disappeared in the Adelaide drug market, one quarter of users participating in the Ecstasy and Related Drugs Reporting System survey reported use of Ecstasy with high and medium purity (Sindicich and Burns, 2010). Also, although our results showed increased methamphetamine use in the population, the Illicit Drug Reporting System actually indicated less prevalence of methamphetamine use among Australian users in 2010 (Stafford and Burns, 2010). These disagreements may be because of the different sampling time and location of the surveys and our study, or they could derive from the different rationales of these two methods. When the Ecstasy and Related Drugs Reporting System participants were asked about their Ecstasy use, it is possible that they did not really know what substance they were taking, whereas the wastewater analysis directly measured the consumption of MDMA. Also, in the above-mentioned Illicit Drug Reporting System report, the sample size was restricted to less than 1,000 people, all of whom were injecting drug users, whereas wastewater samples collected in this study reflected the drug use in the whole population (1.3 million) in the sampling area, irrespective of route of administration.

This study provides direct and timely data to support the reported MDMA decline and suggests that there may be a worldwide shortage of the drug. These data were also supported by roadside saliva testing, which could be regarded as a large-scale survey with random sampling. The data also suggest an increase in methamphetamine use over the same period as the decline in MDMA. Finally, this study confirms the potential of the wastewater analysis approach as an important tool to provide objective and near–real-time information on changes in drug use patterns within communities.

Acknowledgments

The authors gratefully acknowledge the staff in SA Water and United Water for their assistance in sampling and Dr. Andrew Humpage for facilitating the collection of the samples.

References

Elliott, S. (2011). Cat and mouse: The analytical toxicology of designer drugs. *Bioanalysis, 3,* 249–251.

European Monitoring Centre for Drugs and Drug Addiction. (2010a). *Annual report 2010: The state of the drugs problem in Europe.* Lisbon, Portugal: Author.

European Monitoring Centre for Drugs and Drug Addiction. (2010b). *Drugnet Europe 70.* Lisbon, Portugal: Author.

Health Research Board. (2010). *Drugnet Ireland: Alcohol and drug research newsletter, 34.* Retrieved from http://www.hrb.ie/uploads/tx_hrbpublications/Drugnet_34_-published.pdf

Irvine, R. J., Kostakis, C., Felgate, P. D., Jaehne, E. J., Chen, C., & White, J. M. (2011). Population drug use in Australia: A wastewater analysis. *Forensic Science International.* doi:10.1016/j.forsciint.2011.01.037

Sindicich, N., & Burns, L. (2010). An overview of the 2010 EDRS: The regular ecstasy user survey findings. *EDRS Drug Trends Bulletin, October 2010.* Sydney, Australia: National Drug and Alcohol Research Centre, University of New South Wales.

Stafford, J., & Burns, L. (2010). An overview of the 2010 IDRS: The Injecting Drug Users survey key findings. *Drug Trends Bulletin, October 2010.* Sydney, Australia: National Drug and Alcohol Research Centre, University of New South Wales.

United Nations Office on Drugs and Crime. (2010). *World Drug Report 2010.* New York, NY: United Nations Publications.

van Nuijs, A. L. N., Castiglioni, S., Tarcomnicu, I., Postigo, C., de Alda, M. L., Neels, H., . . . Covaci, A. (2010). Illicit drug consumption estimations derived from wastewater analysis: A critical review. *Science of the Total Environment.* doi:10.1016/j.scitotenv.2010.05.030

Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R., & Fanelli, R. (2008). Estimating community drug abuse by wastewater analysis. *Environmental Health Perspectives, 116,* 1027–1032.

Zuccato, E., Chiabrando, C., Castiglioni, S., Calamari, D., Bagnati, R., Schiarea, S., & Fanelli, R. (2005). Cocaine in surface waters: A new evidence-based tool to monitor community drug abuse. *Environmental Health: A Global Access Science Source, 4,* 14.

CHAPTER 5 "Increases in use of novel synthetic stimulant are not directly linked to decreased use of 3,4-methylenedioxy-N-methylamphetamine (MDMA)" (Publication 4)

This study is a follow-up to Chapter 4, which revealed a marked decline in MDMA between 2009 and 2010. It was reported that a group of synthetic stimulants became popular since 2009 (EMCDDA 2010a; EMCDDA 2010b; Health Research Board 2010; UNODC 2010), but a number of unknowns existed, including the prevalence and use pattern. It was also uncertain whether their emergence was associated with the decline in MDMA, or independent of it. Conventional survey methods have significant limitations in obtaining such information, since users are likely to be mistaken as to both the drug and the doses they have used. This is because these drugs are often sold under a variety of street names, which do not accurately reflect their chemical and pharmacological properties.

Comparatively, wastewater analysis is a suitable tool for obtaining this information because it directly targets the drugs or metabolites, regardless of their pharmacological properties or street names. It also has the ability to provide timely data, which is important for a fast-changing drug market.

This study developed analytical methods for the six most reported synthetic stimulants, namely methcathinone, mephedrone, methylone, MDPV, BZP and TFMPP. The developed

methods were then applied to collected wastewater samples to investigate the use pattern and prevalence of these drugs in Adelaide between 2009 and 2011.

As hypothesised, these targeted drugs were quantified in the collected wastewater samples. Data analysis indicated that mephedrone, methylone and BZP were mainly used on weekends, while methcathinone, MDPV and TFMPP use were more constant throughout the week. Interestingly, while some of these novel synthetic stimulants showed increasing prevalence during the decrease of MDMA use, others showed trends independent of MDMA availability. Moreover, geographic and temporal comparisons of the data indicated sporadic rather than constant patterns of novel stimulant use.

The results showed that although there was an increase in synthetic stimulant use during the period of MDMA decline, these two phenomena were not directly related. This further suggested to the authorities that controlling one drug may not necessarily stimulate the use of its alternatives.

Chen, C., Kostakis, C., Irvine, R.J. & white, J.M. (2013) Increases in use of novel synthetic stimulant are not directly linked to decreased use of 3,4-methylenedioxy-N-methylamphetamine (MDMA). *Forensic Science International, v. 231(1-3), pp. 278-283*

NOTE:
This publication is included on pages 87-92 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.1016/j.forsciint.2013.06.007

Supporting materials

Methods for stability and binding tests

Stability tests were carried out in fresh wastewater samples that were stored in different conditions, including: no treatment, storage at 4 °C, −20 °C, acidification, preservative-addition and filtration. Concentrations of analytes was measured on the starting day (day 0) and on days 1, 2, 3, 7 and 14 thereafter to show whether there was significant change. A change of more than 15% was considered significant formation (> 115%) or degradation (< 85%). Stability of analytes in reconstituted extract was evaluated by comparing the analytes' peak areas with a standard solution prepared in ethanol and stored at −20 °C after storage at either 4 °C or −20 °C or 1, 3, 7 and 14 days.

For binding tests, 100 mL sample was spiked with reference standards to ensure that the concentrations of these analytes were above the LOQ of the analytical method. After 2 h, half of the mixed sample was filtered under vacuum using 1.6 µm GF/A glass microfiber filters (Whatman Ltd., Kent, UK), while the other 50 mL remained unfiltered. Analytes in filtered and unfiltered samples were then extracted using liquid-liquid extraction (LLE) and analysed by LC–MS/MS [27]. Data were then compared by paired two-tailed t test using GraphPad Prism™ 5 (GraphPad Software Inc., La Jolla, CA) to assess whether there was significant analyte loss after filtration.

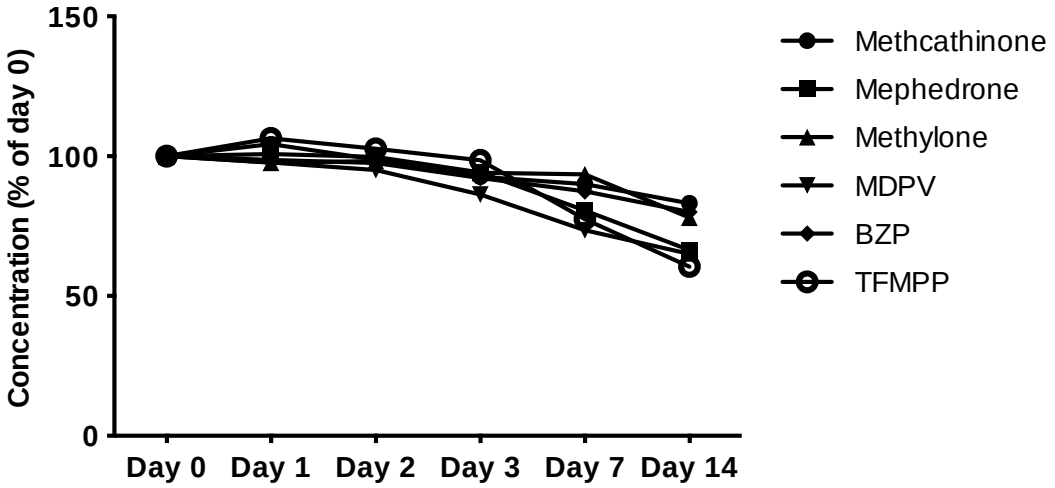


Figure S1. Analyte stability in untreated wastewater in 14 days. Data are expressed as average value of remaining percentages of Day 0, n = 3. Mephedrone, 4-methylmethcathinone; methylone, 3,4-methylenedioxy-N-methylcathinone; MDPV, methylenedioxypyrovalerone; BZP, benzylpiperazine; TFMPP, 3-trifluoromethylphenylpiperazine. Initial concentration was 25 ng/L for all the drugs.

Table S1. Selected mass spectrometric parameters used in the analysis of 3,4-methylenedioxymethamphetamine (MDMA), methcathinone, 4-methylmethcathinone (mephedrone), 3,4-methylenedioxy-N-methylcathinone (methylone), methylenedioxypyrovalerone (MDPV), benzylpiperazine (BZP) and 3-trifluoromethylphenylpiperazine (TFMPP) for Applied Biosystems 4000 Q-Trap™

Transition	Q_1 m/z	Q_3 m/z	Dwell time (ms)	DP[a] (V)	EP[b] (V)	CE[c] (V)	CXP[d] (V)
MDMA 1[#]	194	163	60	50	10	20	30
MDMA 2	194	105	40	50	10	30	30
MDMA 3	194	135	40	50	10	35	30
Methcathinone 1[#]	164	146	80	50	10	20	10
Methcathinone 2	164	130	40	50	10	42	10
Methcathinone 3	164	105	40	50	10	28	10
Mephedrone 1[#]	178	160	80	50	10	20	10
Mephedrone 2	178	144	40	50	10	45	10
Mephedrone 3	178	119	40	50	10	32	10
Methylone 1[#]	208	160	80	50	10	25	10
Methylone 2	208	190	40	50	10	20	10
Methylone 3	208	58	40	50	10	45	10
MDPV 1[#]	276	126	80	70	10	40	10
MDPV 2	276	135	40	70	10	40	10
MDPV 3	276	175	40	70	10	30	10
BZP 1[#]	177	91	80	50	10	35	10
BZP 2	177	65	40	50	10	65	10
BZP 3	177	85	40	50	10	25	10
TFMPP 1[#]	231	188	80	50	10	35	10
TFMPP 2	231	119	40	50	10	45	10
TFMPP 3	231	168	40	50	10	40	10
MDMA-d_5*[#]	199	165	60	50	10	20	30

[a] Declustering potential

[b] Entrance potential

[c] Collision energy

[d] Collision cell exit potential

[#] Transitions used for quantification

* Internal standard

Table S2. Validation data of the SPE-LC-MS/MS method used in this study. MDMA, 3,4-methylenedioxymethamphetamine; Mephedrone, 4-methylmethcathinone; methylone, 3,4-methylenedioxy-N-methylcathinone; MDPV, methylenedioxypyrovalerone; BZP, benzylpiperazine; TFMPP, 3-trifluoromethylphenylpiperazine

	LOQ (ng/L)	Linear range (ng/L)	R^2	Absolute recovery (%; mean ± 95% CI)	Relative recovery (%; mean ± 95% CI)	RSD (%)
MDMA	1	1−1000	0.995	89.3 ± 2.8	100.0 ± 2.1	3.66
Methcathinone	1	1−1000	0.994	98.15 ± 11.42	117.49 ± 9.89	10.76
Mephedrone	1	1−3000	0.993	98.60 ± 3.86	104.27 ± 4.24	4.87
Methylone	1	1−2000	0.995	99.45 ± 1.81	102.38 ± 1.93	2.39
MDPV	1	1−2000	0.992	98.68 ± 1.09	100.04 ± 1.11	1.30
BZP	1	1−2000	0.994	104.60 ± 5.45	92.39 ± 4.84	6.36
TFMPP	1	1−2000	0.998	101.15 ± 1.24	100.78 ± 1.19	1.39

Note: LOQ and linear ranges were determined on “artificial wastewater”

Table S3. Validation data of the LLE-LC-MS/MS method used in the binding tests. MDMA, 3,4-methylenedioxymethamphetamine; Mephedrone, 4-methylmethcathinone; methylone, 3,4-methylenedioxy-N-methylcathinone; MDPV, methylenedioxypyrovalerone; BZP, benzylpiperazine; TFMPP, 3-trifluoromethylphenylpiperazine

	LOQ (ng/L)	Linear range (ng/L)	R^2	Absolute recovery (%; mean ± 95% CI)	Relative recovery (%; mean ± 95% CI)	RSD (%)
MDMA	2	2−1000	0.995	70.74 ± 1.72	91.33 ± 2.21	2.09
Methcathinone	2	2−500	0.990	53.55 ± 6.06	101.04 ± 7.44	14.42
Mephedrone	2	2−2000	0.994	66.83 ± 2.32	99.54 ± 3.66	4.68
Methylone	2	2−1000	0.994	72.07 ± 1.95	97.31 ± 2.76	3.66
MDPV	2	2−1000	0.997	53.11 ± 5.31	111.22 ± 11.19	13.41
BZP	2	2−1000	0.994	35.09 ± 3.45	112.86 ± 10.29	13.01
TFMPP	2	2−1000	1.000	65.28 ± 3.55	101.54 ± 5.80	7.21

Note: LOQ and linear ranges were determined on “artificial wastewater”

CHAPTER 6 Discussion

This thesis contributes to the field of illicit drug control in two ways. Firstly, the method of wastewater analysis is further validated, which improves the reliability and cost effectiveness of the method. Secondly, essential information on population stimulant use is collected in this thesis. This information may assist authorities in controlling population stimulant use, which consequently improves the population health and reduce the social problems associated with illicit stimulant use.

1. Summary of major achievements

1.1. Method validation: evaluation of pre-analysis loss

The work presented in this thesis served to validate wastewater analysis as a tool to investigate population drug use. Firstly, this work showed that cocaine as the parent drug is not a suitable analytical target for the monitoring of population cocaine use due to stability reasons, and its metabolite benzoylecgonine should be used instead. This conclusion was supported by other studies (Gheorghe, van Nuijs et al. 2008; Baker and Kasprzyk-Hordern 2011a; van Nuijs, Abdellati et al. 2012).

Another major finding of this work was that 6-monoacetylmorphine decomposes quickly in untreated wastewater as previously reported (Baker and Kasprzyk-Hordern 2011a; van Nuijs, Abdellati et al. 2012). Additionally, this work showed that acidifying the sample to pH 2 or preserving the sample with 2 g/L $Na_2S_2O_5$ significantly improved the stability of 6-monoacetylmorphine, and suggested that latter is the most practical approach to preserve

samples for 6-monoacetylmorphine analysis. Van Nuijs et al (2009) developed an analytical method for 6-monoacetylmorphine in wastewater, but failed to detect it in authentic samples that were acidified to pH 2 during storage. The authors hypothesised that it might be due to the storage conditions applied in this study. However, the work carried out in this thesis suggested that the storage condition used in their study was sufficient to stabilise 6-monoacetylmorphine in the samples. Hence, the reason that 6-monoacetylmorphine was not detected might be that it decomposed prior to sample collection, or that the original concentration of 6-monoacetylmorphine was below the LOQ of the analytical method applied.

The third contribution of this work was that it confirmed no binding of the studied analytes to particular matter and filter paper, using a more reliable method. Although previous studies showed similar conclusions (Gheorghe, van Nuijs et al. 2008; Metcalfe, Tindale et al. 2010; Baker and Kasprzyk-Hordern 2011a; Baker, Ocenaskova et al. 2012), their methods were based on the analysis of the filtered residues, hence irreversible bindings could not be excluded.

Moreover, this work investigated five different storage conditions as well as the condition of non-treatment. This strategy allowed comparisons among all the conditions to show how the stability is improved by each one, and which condition showed the best efficiency. Based on the result, the most cost-effective storage condition was recommended according to the analyte, which should be applied in future studies.

In addition, this work compared the stability data obtained in this study with those

previously reported, and revealed that stability results may vary between wastewater from different resources. This encourages other research groups to assess analyte stability in their respective matrix instead of using data reported in the literature if the percentage of loss is required for back-calculation.

The rationale and method of this study is applicable to other compounds in wastewater, and can hence serve as a template for similar studies in the future. As an example, similar experiments were carried out in Chapter 5 of this thesis when validating the analytical method for the novel synthetic stimulants.

1.2. Application in South Australia

A major aim of the project was to apply the validated method of wastewater analysis to a major South Australian city with a population of 1.3 million, as well as to 10 smaller regional centres with populations varying between 370 and 23300 (census data).

This study provided direct evidence showing that stimulant use is higher on weekends, which is supported by other wastewater studies (Karolak, Nefau et al. 2010; Terzic, Senta et al. 2010). This is not surprising considering the stimulants are often used at parties and in nightclubs (Topp, Breen et al. 2004). However, the fact that MDMA use on weekends is 5 times higher than weekdays indicated that MDMA use follows a slightly different pattern from methamphetamine, which was not reflected in surveys (White, Vial et al. 2008).

With regards to geographical comparisons, MDMA use in regional areas was higher than metropolitan areas. Considering MDMA is a popular “party drug” (Topp, Breen et al.

2004), this result suggested that MDMA may be used more often in private parties in remote areas instead of public bars and clubs concentrating in metropolitan areas. This finding contradicts the World Drug Report (UNODC 2012) and other wastewater studies (Banta-Green, Field et al. 2009; Karolak, Nefau et al. 2010) showing that MDMA level was higher in metropolitan areas. However, the study carried out by Banta-Green et al also indicated higher use of MDMA in small rural towns than in large ones. Together, these studies suggest that urbanisation level may not be directly associated with MDMA prevalence.

Moreover, international comparisons suggested that the variation between different countries in terms of the preference for specific illicit stimulants may have been underestimated by the World Drug Reports (UNODC 2008; UNODC 2009).

1.3. Monitoring of MDMA use

Although a decrease in MDMA use has been reported based on surveys and seizure data (EMCDDA 2010b; UNODC 2010), this study provided direct evidence of the decline during the period 2009–2010, and suggested that it extended beyond Europe.

From 1998 to 2007, when use of MDMA increased in Australia, the use of methamphetamine decreased (UNODC 2010). Hence it was important to know whether there was an increase in use of methamphetamine during the recent decline in MDMA use; in other words, whether the use of these two stimulants is correlated. The answer to this question is important in understanding the population behaviour in stimulant use. Data in this study showed that there was an increase in methamphetamine use during the same

period of MDMA decline. This suggested that MDMA and methamphetamine use may be associated. Cocaine use in the population was not found to be associated with MDMA use, suggesting different user groups of these two stimulants.

The reason for the marked decline in MDMA use was uncertain, but reports suggested that strengthening of international laws since late 2008 and some large seizures of MDMA precursors may have played an important role (UNODC 2010). If this hypothesis was correct, this study served as the first example of using wastewater analysis as an objective and quick approach to evaluate the effectiveness of drug control campaigns.

1.4. Novel synthetic stimulants in wastewater

During the decline in MDMA use, some other reports suggested that MDMA might have been replaced by novel synthetic stimulants (EMCDDA 2010a; EMCDDA 2010b; Health Research Board 2010; UNODC 2010). However, the investigation in this thesis found that although sporadic and localised increases of other stimulants were seen, a population-scale replacement of MDMA by the novel synthetic stimulants did not occur, which contradicted the hypothesis. It was possible that during the decline in MDMA use, users were searching for alternatives. However, the novel synthetic drugs did not meet the users' expectations or were not readily available and hence were not widely used. This hypothesis was supported by a survey on mephedrone showing that MDMA is preferred by a majority of "ecstasy" users and that the popularity of mephedrone was driven by factors related to its legality and the unreliable purity of MDMA, rather than its pharmacological effects (Carhart-Harris, King et al. 2011). Based on this hypothesis, the use of MDMA is likely to re-emerge once

the causes of its decline (the shortage in supply) no longer exist. Unpublished data obtained by our group support this hypothesis by showing that MDMA consumption in Adelaide returned to the 2009 level in late 2011. To summarise, controlling the precursors of MDMA may have significantly reduced the use of MDMA in the population, without triggering large increase in use of alternatives. However, the drug control policies on MDMA should be continuous if long-term effects are expected.

From a technical perspective, although similar methods have been reported (Baker and Kasprzyk-Hordern 2011b; van Nuijs, Gheorghe et al. 2013), this study reported a new SPE-LC-MS/MS method with more supporting information (e.g. stability data). This is also the first study to develop the analytical method for methylone in wastewater, and the first monitoring program for these novel synthetic stimulants in the world.

2. Conclusion

Using wastewater samples collected in South Australia, this thesis presented data which not only are valuable to the Australian authorities, but also have international implications. Firstly, the method of wastewater analysis was further validated by assessing whether there was pre-analysis loss of analytes, and how to reduce it if so. Secondly, this thesis provided data on the pattern of population stimulant use, which is not available via traditional monitoring methods. It also revealed that MDMA use can change quickly and markedly in the market, with or without links to changes in other stimulant use.

3. Future research

The preferable strategy for wastewater sampling is flow-dependent sampling with short (< 5 min) intervals (Ort, Lawrence et al. 2010). However, for the purpose of this study, the sampling intervals in each 24-hour cycle were about 10–15 min, and regional samples were grabbed. In addition, samples were only collected from the State of South Australia, and in some instances only from metropolitan areas of Adelaide. With more acceptance of the wastewater epidemiology approach, it is likely that high frequency, flow-dependent sampling will be possible for more WWTPs in future studies.

The accuracy of population drug use estimation remains a significant limitation. Census data cannot be relied upon to provide an accurate picture of population demographics on a day-to-day basis. Flow measurement is another major error source for back-calculation (Lai, Ort et al. 2011). These issues can potentially be solved by introducing a population biomarker, which is an endogenous substance or something excreted by a large proportion of the population (Chiaia, Banta-Green et al. 2008). By comparing the concentrations of analytes to the co-analysed population biomarker, the prevalence of drug use in the population can be estimated without knowing the census population or the wastewater flow (Lai, Ort et al. 2011). However, finding a suitable population biomarker can be difficult since a number of criteria (detectability, representativeness, stability, etc.) need to be met. A number of potential population biomarkers such as creatinine (Chiaia, Banta-Green et al. 2008), coprostanol (Daughton 2012a), atenolol (Lai, Ort et al. 2011), and concentrations of nitrogen, phosphorus and oxygen in wastewater (van Nuijs, Mougel et al. 2011) have been

suggested, but their usefulness have not been fully evaluated.

Metabolic profiles of a studied drug in humans are also crucial pieces of information in estimating drug consumption. For the novel stimulants this information is largely unknown. Even for the popular drugs which have been investigated, the representativeness to the user population is unknown, as previously discussed in Section 5.5, Chapter 1. An adjusted excretion ratio, which takes into account all the factors and better represents the user population, needs to be used for a more accurate estimation of drug consumption in future.

In view of the rapidly expanding market in synthetic stimulants, the range of compounds may be extended beyond what was covered in the current work. Furthermore, analytical methods for other groups of illicit and licit drugs or other health-related substances such as disease biomarkers (Daughton 2012b) may also be included to gain more information on population health. The studies presented in this thesis can serve as examples and foundations for such future work.

BIBLIOGRAPHY

Abt Associates Inc. (2001). What America's Users Spend on Illegal Drugs 1988-2000.

Abt Associates Inc. (2011). ADAM II: 2010 Annual Report.

Advisory Council on the Misuse of Drugs (2011). Consideration of the Novel Psychoactive Substances ('Legal Highs'). London.

American Psychiatric Association (1994). Diagnostic and Statistical Manual of Mental Disorders, 4th ed. Washington, D. C., American Psychiatric Association.

Antia, U., Lee, H. S., Kydd, R. R., Tingle, M. D. and Russell, B. R. (2009). Pharmacokinetics of 'party pill' drug N-benzylpiperazine (BZP) in healthy human participants. *Forensic Science International* 186(1–3): 63-67.

Antia, U., Tingle, M. D. and Russell, B. R. (2009). 'Party pill' drugs–BZP and TFMPP. *New Zealand Medical Journal* 122(1307): 55-68.

Antia, U., Tingle, M. D. and Russell, B. R. (2010). Validation of an LC-MS method for the detection and quantification of BZP and TFMPP and their hydroxylated metabolites in human plasma and its application to the pharmacokinetic study of TFMPP in humans. *Journal of Forensic Sciences* 55(5): 1311-1318.

Austin, H. and Monasterio, E. (2004). Acute psychosis following ingestion of 'Rapture'. *Australasian Psychiatry* 12(4): 406-408.

Australian Crime Commission (2009). Australian Crime Commission Illicit Drug Data Report 2007–2008.

Australian Federal Police (2004). Australian Federal Police Drug Harm Index 2004.

Australian Institute of Health and Welfare (2008). 2007 National Drug Strategy Household Survey: first results. Canberra, AIHW.

Australian Institute of Health and Welfare (2011). 2010 National Drug Strategy Household Survey Report. Canberra, AIHW.

Baba, T., Yamada, H., Oguri, K. and Yoshimura, H. (1987). A new metabolite of methamphetamine; evidence for formation of N-[(1-methyl-2-phenyl)ethyl]ethanimine N-oxide. *Xenobiotica* 17(9): 1029-1038.

Baker, D. R. and Kasprzyk-Hordern, B. (2011a). Critical evaluation of methodology commonly used in sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography-mass spectrometry. *Journal of Chromatography A* 1218(44): 8036-8059.

Baker, D. R. and Kasprzyk-Hordern, B. (2011b). Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography-positive electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A* 1218(12): 1620-1631.

Baker, D. R., Ocenaskova, V., Kvicalova, M. and Kasprzyk-Hordern, B. (2012). Drugs of abuse in wastewater and suspended particulate matter--further developments in sewage epidemiology. *Environment International* 48: 28-38.

Banta-Green, C. J., Field, J. A., Chiaia, A. C., Sudakin, D. L., Power, L. and de Montigny, L. (2009). The spatial epidemiology of cocaine, methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) use: a demonstration using a population measure of community drug load derived from municipal wastewater. *Addiction* 104(11): 1874-1880.

Barash, P. G. (1977). Cocaine in Clinical Medicine. *NIDA Research Monograph*. 13: 202-209.

Bartelt-Hunt, S. L., Snow, D. D., Damon, T., Shockley, J. and Hoagland, K. (2009). The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface

waters in Nebraska. *Environmental Pollution* 157(3): 786-791.

Baumann, M. H., Partilla, J. S., Lehner, K. R., Thorndike, E. B., Hoffman, A. F., Holy, M., et al. (2013). Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive 'bath salts' products. *Neuropsychopharmacology* 38(4): 552-562.

Berman, S. M., Kuczenski, R., McCracken, J. T. and London, E. D. (2009). Potential adverse effects of amphetamine treatment on brain and behavior: a review. *Molecular Psychiatry* 14(2): 123-142.

Bernschneider-Reif, S., Oxler, F. and Freudenmann, R. W. (2006). The origin of MDMA ("ecstasy")–separating the facts from the myth. *Pharmazie* 61(11): 966-972.

Bhattachary, S. and Powell, J. H. (2001). Recreational use of 3,4-methylenedioxymethamphetamine (MDMA) or 'ecstasy': evidence for cognitive impairment. *Psychological Medicine* 31(4): 647-658.

Bijlsma, L., Sancho, J. V., Pitarch, E., Ibanez, M. and Hernandez, F. (2009). Simultaneous ultra-high-pressure liquid chromatography -tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis metabolite in surface water and urban wastewater. *Journal of Chromatography A* 1216(15): 3078-3089.

Bisceglia, K. J. (2010). Occurence and fate of pharmaceuticals, illicit drugs, and other emerging contaminants in natural and engineered environments. Baltimore, The Johns Hopkins University. Ph.D.: 397.

Blum, K., Chen, A. L., Giordano, J., Borsten, J., Chen, T. J., Hauser, M., et al. (2012). The addictive brain: all roads lead to dopamine. *Journal of Psychoactive Drugs* 44(2): 134-143.

Boehringer Ingelheim (1969). α-Substituted-ketones and processes for their preparation. The Patent Office London. UK. GB 1149366.

Boleda, M. A., Galceran, M. A. and Ventura, F. (2009). Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. *Water Research* 43(4): 1126-1136.

Boleda, M. R., Galceran, M. T. and Ventura, F. (2007). Trace determination of cannabinoids and opiates in wastewater and surface waters by ultra-performance liquid chromatography -tandem mass spectrometry. *Journal of Chromatography A* 1175(1): 38-48.

Bolla, K. I., McCann, U. D. and Ricaurte, G. A. (1998). Memory impairment in abstinent MDMA ("Ecstasy") users. *Neurology* 51(6): 1532-1537.

Bones, J., Thomas, K. V. and Paull, B. (2007). Using environmental analytical data to estimate levels of community consumption of illicit drugs and abused pharmaceuticals. *Journal of Environmental Monitoring* 9(7): 701-707.

Brennan, K., Johnstone, A., Fitzmaurice, P., Lea, R. and Schenk, S. (2007). Chronic benzylpiperazine (BZP) exposure produces behavioral sensitization and cross-sensitization to methamphetamine (MA). *Drug and Alcohol Dependence* 88(2-3): 204-213.

Cadet, J. L., Ali, S. F., Rothman, R. B. and Epstein, C. J. (1995). Neurotoxicity, drugs and abuse, and the CuZn-superoxide dismutase transgenic mice. *Molecular Neurobiology* 11(1-3): 155-163.

Caldwell, J., Dring, L. G. and Williams, R. T. (1972). Metabolism of (14 C)methamphetamine in man, the guinea pig and the rat. *Biochemical Journal* 129(1): 11-22.

Cameron, K. N., Kolanos, R., Solis, E., Jr., Glennon, R. A. and De Felice, L. J. (2013). Bath salts components mephedrone and methylenedioxypyrovalerone (MDPV) act synergistically at the human dopamine transporter. *British Journal of Pharmacology* 168(7): 1750-1757.

Campbell, H., Cline, W., Evans, M., Lloyd, J. and Peck, A. W. (1973). Comparison of the effects of dexamphetamine and 1-benzylpiperazine in former addicts. *European Journal of Clinical Pharmacology* 6(3): 170-176.

Carhart-Harris, R. L., King, L. A. and Nutt, D. J. (2011). A web-based survey on mephedrone. *Drug and Alcohol Dependence* 118(1): 19-22.

Carrera, M. R., Meijler, M. M. and Janda, K. D. (2004). Cocaine pharmacology and current pharmacotherapies for its abuse. *Bioorganic & Medicinal Chemistry* 12(19): 5019-5030.

Castiglioni, S., Bagnati, R., Melis, M., Panawennage, D., Chiarelli, P., Fanelli, R., et al. (2011). Identification of cocaine and its metabolites in urban wastewater and comparison with the human excretion profile in urine. *Water Research* 45(16): 5141-5150.

Castiglioni, S., Zuccato, E., Chiabrando, C., Fanelli, R. and Bagnati, R. (2008). Mass spectrometric analysis of illicit drugs in wastewater and surface water. *Mass Spectrometry Reviews* 27(4): 378-394.

Castiglioni, S., Zuccato, E., Crisci, E., Chiabrando, C., Fanelli, R. and Bagnati, R. (2006). Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography- tandem mass spectrometry. *Analytical Chemistry* 78(24): 8421-8429.

Chen, C., Kostakis, C., Harpas, P., Felgate, P. D., Irvine, R. J. and White, J. M. (2011). Marked decline in 3,4-methylenedioxymethamphetamine (MDMA) based on wastewater analysis. *Journal of Studies on Alcohol and Drugs* 72(5): 737-740.

Chen, W. J., Fang, C. C., Shyu, R. S. and Lin, K. C. (2006). Underreporting of illicit drug use by patients at emergency departments as revealed by two-tiered urinalysis. *Addictive Behaviors* 31(12): 2304-2308.

Chiaia, A. C., Banta-Green, C. and Field, J. (2008). Eliminating solid phase extraction with large-volume injection LC/MS/MS: analysis of illicit and legal drugs and human urine

indicators in U.S. wastewaters. *Environmental Science & Technology* 42(23): 8841-8848.

Cohen, B. M. and Butler, R. (2011). BZP-party pills: a review of research on benzylpiperazine as a recreational drug. *International Journal of Drug Policy* 22(2): 95-101.

Cole, J. C. and Sumnall, H. R. (2003). The pre-clinical behavioural pharmacology of 3,4-methylenedioxymethamphetamine (MDMA). *Neuroscience & Biobehavioral Reviews* 27(3): 199-217.

Collins, D. J. and Lapsley, H. M. (2008). The costs of tobacco, alcohol and illicit drug abuse to Australian society in 2004/05.

Connor, T. J., McNamara, M. G., Finn, D., Currid, A., O'Malley, M., Redmond, A. M., et al. (1998). Acute 3,4-methylenedioxymethamphetamine(MDMA) administration produces a rapid and sustained suppression of immune function in the rat. *Immunopharmacology* 38(3): 253-260.

Cook, C. E., Jeffcoat, A. R., Hill, J. M., Pugh, D. E., Patetta, P. K., Sadler, B. M., et al. (1993). Pharmacokinetics of methamphetamine self-administered to human subjects by smoking S-(+)-methamphetamine hydrochloride. *Drug Metabolism and Disposition* 21(4): 717-723.

Cook, C. E., Jeffcoat, A. R., Sadler, B. M., Hill, J. M., Voyksner, R. D., Pugh, D. E., et al. (1992). Pharmacokinetics of oral methamphetamine and effects of repeated daily dosing in humans. *Drug Metabolism and Disposition* 20(6): 856-862.

Cozzi, N. V., Sievert, M. K., Shulgin, A. T., Jacob, P., 3rd and Ruoho, A. E. (1999). Inhibition of plasma membrane monoamine transporters by beta-ketoamphetamines. *European Journal of Pharmacology* 381(1): 63-69.

Creighton, F. J., Black, D. L. and Hyde, C. E. (1991). 'Ecstasy' psychosis and flashbacks. *British Journal of Psychiatry* 159: 713-715.

Crick, S. J., Sheppard, M. N. and Anderson, R. H. (2000). Neural supply of the heart. *The Nervous System and the Heart*. Horst, G.J.t. Totowa, Humana Press: 3-54.

Cruickshank, C. C. and Dyer, K. R. (2009). A review of the clinical pharmacology of methamphetamine. *Addiction* 104(7): 1085-1099.

Dal Cason, T. A., Young, R. and Glennon, R. A. (1997). Cathinone: an investigation of several N-alkyl and methylenedioxy-substituted analogs. *Pharmacology Biochemistry and Behavior* 58(4): 1109-1116.

Dargan, P. I., Sedefov, R., Gallegos, A. and Wood, D. M. (2011). The pharmacology and toxicology of the synthetic cathinone mephedrone (4-methylmethcathinone). *Drug Testing and Analysis* 3(7-8): 454-463.

Daughton, C. G. (2001). Illicit Drugs in Municipal Sewage: Proposed New Non-Intrusive Tool to Heighten Public Awareness of Societal Use of Illicit/Abused Drugs and Their Potential for Ecological Consequences. *Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issue.* Jones-Lepp, T.L. Washington, D. C., American Chemical Society: 348-364.

Daughton, C. G. (2012a). Real-time estimation of small-area populations with human biomarkers in sewage. *Science of the Total Environment* 414: 6-21.

Daughton, C. G. (2012b). Using biomarkers in sewage to monitor community-wide human health: Isoprostanes as conceptual prototype. *Science of the Total Environment* 424(0): 16-38.

de Boer, D., Bosman, I. J., Hidvegi, E., Manzoni, C., Benko, A. A., dos Reys, L. J., et al. (2001). Piperazine-like compounds: a new group of designer drugs-of-abuse on the European market. *Forensic Science International* 121(1-2): 47-56.

de la Torre, R., Farre, M., Roset, P. N., Pizarro, N., Abanades, S., Segura, M., et al. (2004). Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition.

Therapeutic Drug Monitoring 26(2): 137-144.

Drew, C. D., Knight, G. T., Hughes, D. T. and Bush, M. (1978). Comparison of the effects of D-(-)-ephedrine and L-(+)-pseudoephedrine on the cardiovascular and respiratory systems in man. *British Journal of Clinical Pharmacology* 6(3): 221-225.

Drug Enforcement Administration (2012). N-benzylpiperazine (Street Names: BZP, A2, Legal E or Legal X).

Emerson, T. S. and Cisek, J. E. (1993). Methcathinone: a Russian designer amphetamine infiltrates the rural midwest. *Annals of Emergency Medicine* 22(12): 1897-1903.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2002). Handbook for surveys on drug use among the general population.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2009). Methamphetamine: a European Union perspective in the global context.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2010a). Annual report 2010: the state of the drugs problem in Europe.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2010b). Drugnet Europe 70. Lisbon, EMCDDA.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2010c). Drugnet Europe 71.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2012). Annual report 2012.

Farrar, H. C. and Kearns, G. L. (1989). Cocaine: clinical pharmacology and toxicology. *Journal of Pediatrics* 115(5): 665-675.

Fotiou, F., Fountoulakis, K. N., Goulas, A., Alexopoulos, L. and Palikaras, A. (2000). Automated standardized pupillometry with optical method for purposes of clinical practice

and research. *Clinical Physiology* 20(5): 336-347.

Frost, N., Griffiths, P. and Fanelli, R. (2008). Peering into dirty waters: the potential and implications of a new approach to monitoring drug consumption. *Addiction* 103(8): 1239-1241.

Fuller, R. W. and Mason, N. R. (1981). Structure-Activity Relationships in the Actions of 1-Phenyl-Piperazines on Brain Serotonin Receptors. *Serotonin: Current Aspects of Neurochemistry and Function*. Haber, B., Gabay, S., Issidorides, M.R. and Alivisatos, S.G.A. New York, Springer: 359-368.

Gaffney, A., Jones, W., Sweeney, J. and Payne, J. (2010). Drug use monitoring in Australia: 2008 annual report on drug use among police detainees-monitoring report No. 9. Canberra, Australian Institute of Criminology.

Gawin, F. H. and Ellinwood, E. H., Jr. (1988). Cocaine and other stimulants. Actions, abuse, and treatment. *New England Journal of Medicine* 318(18): 1173-1182.

Gawin, F. H., Kleber, H. D., Byck, R., Rounsaville, B. J., Kosten, T. R., Jatlow, P. I., et al. (1989). Desipramine facilitation of initial cocaine abstinence. *Archives of General Psychiatry* 46(2): 117-121.

Gerra, G., Zaimovic, A., Giucastro, G., Maestri, D., Monica, C., Sartori, R., et al. (1998). Serotonergic function after (+/–)3,4-methylene-dioxymethamphetamine ('Ecstasy') in humans. *International Clinical Psychopharmacology* 13(1): 1-9.

Gheorghe, A., van Nuijs, A., Pecceu, B., Bervoets, L., Jorens, P. G., Blust, R., et al. (2008). Analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography-ion trap tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* 391(4): 1309-1319.

Gonzalez-Marino, I., Quintana, J. B., Rodriguez, I. and Cela, R. (2010). Determination of drugs of abuse in water by solid-phase extraction, derivatisation and gas

chromatography-ion trap-tandem mass spectrometry. *Journal of Chromatography A* 1217(11): 1748-1760.

Gonzalez-Marino, I., Quintana, J. B., Rodriguez, I., Rodil, R., Gonzalez-Penas, J. and Cela, R. (2009). Comparison of molecularly imprinted, mixed-mode and hydrophilic balance sorbents performance in the solid-phase extraction of amphetamine drugs from wastewater samples for liquid chromatography -tandem mass spectrometry determination. *Journal of Chromatography A* 1216(48): 8435-8441.

Gordon, L., Tinsley, L., Godfrey, C. and Parrott, S. (2006). The economic and social costs of Class A drug use in England and Wales, 2003/04. Measuring Different Aspects of Problem Drug Use: Methodological Developments. Singleton, N., Murray, R. and Tinsley, L.

Green, A. R., Mechan, A. O., Elliott, J. M., O'Shea, E. and Colado, M. I. (2003). The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacological Reviews* 55(3): 463-508.

Griffith, J. D., Cavanaugh, J., Held, J. and Oates, J. A. (1972). Dextroamphetamine: Evaluation of psychomimetic properties in man. *Archives of General Psychiatry* 26(2): 97-100.

Harris, J. E. and Baldessarini, R. J. (1973). Uptake of (3H)-catecholamines by homogenates of rat corpus striatum and cerebral cortex: effects of amphetamine analogues. *Neuropharmacology* 12(7): 669-679.

Hatch, O. G. (1997). Review of the National Drug Control Strategy: Congressional Hearing. Washinton, D.C. , U.S. Government Printing Office.

Health Research Board (2010). Drugnet Ireland: alcohol and drug research newsletter. Dublin, HRB. Issue 34.

Hoyumpa, A. M., Jr. (1984). Alcohol interactions with benzodiazepines and cocaine.

Advances in Alcohol and Substance Abuse 3(4): 21-34.

Huerta-Fontela, M., Galceran, M. T., Martin-Alonso, J. and Ventura, F. (2008). Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain. *Science of the Total Environment* 397(1-3): 31-40.

Huerta-Fontela, M., Galceran, M. T. and Ventura, F. (2007). Ultraperformance liquid chromatography-tandem mass spectrometry analysis of stimulatory drugs of abuse in wastewater and surface waters. *Analytical Chemistry* 79(10): 3821-3829.

Hummel, D., Loffler, D., Fink, G. and Ternes, T. A. (2006). Simultaneous determination of psychoactive drugs and their metabolites in aqueous matrices by liquid chromatography mass spectrometry. *Environmental Science & Technology* 40(23): 7321-7328.

Hyde, J. F., Browning, E. and Adams, R. (1928). Synthetic homologs of d,l-ephedrine. *Journal of the American Chemical Society* 50(8): 2287-2292.

Inoue, H., Ikeda, N., Kudo, K., Ishida, T., Terada, M. and Matoba, R. (2006). Methamphetamine-related sudden death with a concentration which was of a 'toxic level'. *Legal Medicine (Tokyo)* 8(3): 150-155.

International opium commission (1909). Report of the International opium commission, Shanghai, China, February 1 to February 26. Shanghai.

Jansen, K. L. (1999). Ecstasy (MDMA) dependence. *Drug and Alcohol Dependence* 53(2): 121-124.

Javaid, J. I., Musa, M. N., Fischman, M., Schuster, C. R. and Davis, J. M. (1983). Kinetics of cocaine in humans after intravenous and intranasal administration. *Biopharmaceutics & Drug Disposition* 4(1): 9-18.

Jiggens, J. (2008). Australian heroin seizures and the causes of the 2001 heroin shortage. *International Journal of Drug Policy* 19(4): 273-278.

Johnston, L. D. (2000). General Population Surveys of Drug Abuse. *Guide to Drug Abuse Epidemiology*. Geneva, World Health Organization.

Jones-Lepp, T. L., Alvarez, D. A., Petty, J. D. and Huckins, J. N. (2004). Polar Organic Chemical Integrative Sampling and Liquid Chromatography–Electrospray/Ion-Trap Mass Spectrometry for Assessing Selected Prescription and Illicit Drugs in Treated Sewage Effluents. *Archives of Environmental Contamination and Toxicology* 47(4): 427-439.

Kamata, H. T., Shima, N., Zaitsu, K., Kamata, T., Miki, A., Nishikawa, M., et al. (2006). Metabolism of the recently encountered designer drug, methylone, in humans and rats. *Xenobiotica* 36(8): 709-723.

Karolak, S., Nefau, T., Bailly, E., Solgadi, A. and Levi, Y. (2010). Estimation of illicit drugs consumption by wastewater analysis in Paris area (France). *Forensic Science International* 200(1-3): 153-160.

Kasprzyk-Hordern, B., Dinsdale, R. M. and Guwy, A. J. (2008a). Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography -electrospray tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* 391(4): 1293-1308.

Kasprzyk-Hordern, B., Dinsdale, R. M. and Guwy, A. J. (2008b). The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water Research* 42(13): 3498-3518.

Kasprzyk-Hordern, B., Dinsdale, R. M. and Guwy, A. J. (2009a). Illicit drugs and pharmaceuticals in the environment--forensic applications of environmental data. Part 1: Estimation of the usage of drugs in local communities. *Environmental Pollution* 157(6): 1773-1777.

Kasprzyk-Hordern, B., Dinsdale, R. M. and Guwy, A. J. (2009b). The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during

wastewater treatment and its impact on the quality of receiving waters. *Water Research* 43(2): 363-380.

Kehr, J., Ichinose, F., Yoshitake, S., Goiny, M., Sievertsson, T., Nyberg, F., et al. (2011). Mephedrone, compared with MDMA (ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of awake rats. *British Journal of Pharmacology* 164(8): 1949-1958.

Khan, U. and Nicell, J. A. (2011). Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy. *Environment International* 13(7): 1236-1252.

Khan, U. and Nicell, J. A. (2012). Sewer epidemiology mass balances for assessing the illicit use of methamphetamine, amphetamine and tetrahydrocannabinol. *Science of the Total Environment* 421-422: 144-162.

Knolle, H. (1999). The heroin/cocaine epidemic in Switzerland 1979-1997: a mathematical analysis of law enforcement data. *Substance Use & Misuse* 34(8): 1117-1136.

Kobeissy, F. H., Jeung, J. A., Warren, M. W., Geier, J. E. and Gold, M. S. (2008). Changes in leptin, ghrelin, growth hormone and neuropeptide-Y after an acute model of MDMA and methamphetamine exposure in rats. *Addiction Biology* 13(1): 15-25.

Koesters, S. C., Rogers, P. D. and Rajasingham, C. R. (2002). MDMA ('ecstasy') and other 'club drugs'. The new epidemic. *Pediatric Clinics of North America* 49(2): 415-433.

Lai, F. Y., Ort, C., Gartner, C., Carter, S., Prichard, J., Kirkbride, P., et al. (2011). Refining the estimation of illicit drug consumptions from wastewater analysis: Co-analysis of prescription pharmaceuticals and uncertainty assessment. *Water Research* 45: 4437-4448.

Leonardi, E. T. and Azmitia, E. C. (1994). MDMA (ecstasy) inhibition of MAO type A and type B: comparisons with fenfluramine and fluoxetine (Prozac). *Neuropsychopharmacology* 10(4): 231-238.

Lim, H. K. and Foltz, R. L. (1988). In vivo and in vitro metabolism of

3,4-(methylenedioxy)methamphetamine in the rat: identification of metabolites using an ion trap detector. *Chemical Research in Toxicology* 1(6): 370-378.

Lim, H. K. and Foltz, R. L. (1991a). In vivo formation of aromatic hydroxylated metabolites of 3,4-(methylenedioxy)methamphetamine in the rat: identification by ion trap tandem mass spectrometric (MS/MS and MS/MS/MS) techniques. *Biological Mass Spectrometry* 20(11): 677-686.

Lim, H. K. and Foltz, R. L. (1991b). Ion trap tandem mass spectrometric evidence for the metabolism of 3,4-(methylenedioxy)methamphetamine to the potent neurotoxins 2,4,5-trihydroxymethamphetamine and 2,4,5-trihydroxyamphetamine. *Chemical Research in Toxicology* 4(6): 626-632.

Lin, J. C., Jan, R. K., Lee, H., Jensen, M. A., Kydd, R. R. and Russell, B. R. (2011). Determining the subjective and physiological effects of BZP combined with TFMPP in human males. *Psychopharmacology (Berl)* 214(3): 761-768.

Lin, L. Y., Di Stefano, E. W., Schmitz, D. A., Hsu, L., Ellis, S. W., Lennard, M. S., et al. (1997). Oxidation of methamphetamine and methylenedioxymethamphetamine by CYP2D6. *Drug Metabolism and Disposition* 25(9): 1059-1064.

Logan, B. K., Fligner, C. L. and Haddix, T. (1998). Cause and manner of death in fatalities involving methamphetamine. *Journal of Forensic Sciences* 43(1): 28-34.

Loganathan, B., Phillips, M., Mowery, H. and Jones-Lepp, T. L. (2009). Contamination profiles and mass loadings of macrolide antibiotics and illicit drugs from a small urban wastewater treatment plant. *Chemosphere* 75(1): 70-77.

MacCoun, R. J. and Reuter, P. (2001). Drug War Heresies: Learning from Other Vices, Times, and Places. Cambridge, Cambridge University Press.

Mari, F., Politi, L., Biggeri, A., Accetta, G., Trignano, C., Di Padua, M., et al. (2009). Cocaine and heroin in waste water plants: a 1-year study in the city of Florence, Italy.

Forensic Science International 189(1-3): 88-92.

Martin, L. L. and Sanders-Bush, E. (1982). Comparison of the pharmacological characteristics of 5 HT1 and 5 HT2 binding sites with those of serotonin autoreceptors which modulate serotonin release. *Naunyn-Schmiedeberg's Archives of Pharmacology* 321(3): 165-170.

McCann, U. D. and Ricaurte, G. A. (1991). Lasting neuropsychiatric sequelae of (+-)methylenedioxymethamphetamine ('ecstasy') in recreational users. *Journal of Clinical Psychopharmacology* 11(5): 302-305.

McCann, U. D., Slate, S. O. and Ricaurte, G. A. (1996). Adverse reactions with 3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy'). *Drug Safety* 15(2): 107-115.

McCann, U. D., Szabo, Z., Scheffel, U., Dannals, R. F. and Ricaurte, G. A. (1998). Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. *Lancet* 352(9138): 1433-1437.

Metcalfe, C., Tindale, K., Li, H., Rodayan, A. and Yargeau, V. (2010). Illicit drugs in Canadian municipal wastewater and estimates of community drug use. *Environmental Pollution* 158(10): 3179-3185.

Meyer, M. R., Du, P., Schuster, F. and Maurer, H. H. (2010). Studies on the metabolism of the alpha-pyrrolidinophenone designer drug methylenedioxy-pyrovalerone (MDPV) in rat and human urine and human liver microsomes using GC-MS and LC-high-resolution MS and its detectability in urine by GC-MS. *Journal of Mass Spectrometry* 45(12): 1426-1442.

Meyer, M. R., Wilhelm, J., Peters, F. T. and Maurer, H. H. (2010). Beta-keto amphetamines: studies on the metabolism of the designer drug mephedrone and toxicological detection of mephedrone, butylone, and methylone in urine using gas chromatography-mass spectrometry. *Analytical and Bioanalytical Chemistry* 397(3): 1225-1233.

Mills, E. M., Rusyniak, D. E. and Sprague, J. E. (2004). The role of the sympathetic

nervous system and uncoupling proteins in the thermogenesis induced by 3,4-methylenedioxymethamphetamine. *Journal of Molecular Medicine (Berlin)* 82(12): 787-799.

Moody, D. E., Fang, W. B., Morrison, J. and McCance-Katz, E. (2011). Gender differences in pharmacokinetics of maintenance dosed buprenorphine. *Drug and Alcohol Dependence* 118(2-3): 479-483.

Morgan, M. J. (1999). Memory deficits associated with recreational use of "ecstasy" (MDMA). *Psychopharmacology* 141(1): 30-36.

Nagai, F., Nonaka, R. and Satoh Hisashi Kamimura, K. (2007). The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. *European Journal of Pharmacology* 559(2-3): 132-137.

National Drug and Alcohol Research Centre (2007). National Drug and Alcohol Research Centre 2007 Annual Report. Sydney.

National Drug and Alcohol Research Centre (2009). National Illicit Drug Indicators Project.

National Institute on Drug Abuse (2006). A brife history of MDMA.

Ort, C., Lawrence, M. G., Reungoat, J. and Mueller, J. F. (2010). Sampling for PPCPs in wastewater systems: Comparison of different sampling modes and optimization strategies. *Environmental Science & Technology* 44(16): 6289-6296.

Parrott, A. C. and Lasky, J. (1998). Ecstasy (MDMA) effects upon mood and cognition: before, during and after a Saturday night dance. *Psychopharmacology* 139(3): 261-268.

Paul, B. D. and Cole, K. A. (2001). Cathinone (Khat) and methcathinone (CAT) in urine specimens: a gas chromatographic-mass spectrometric detection procedure. *Journal of Analytical Toxicology* 25(7): 525-530.

Pedersen, A. J., Reitzel, L. A., Johansen, S. S. and Linnet, K. (2012). In vitro metabolism studies on mephedrone and analysis of forensic cases. *Drug Testing and Analysis*.

Petersen, R. C. (1977). History of cocaine. *NIDA Research Monograph*. Series 13: 17-34.

Peyton, J. and Shulgin, A. (1996). Novel N-substituted-2-amino-3',4'-methylene-dioxypropiophenones.

Postigo, C., Lopez de Alda, M. J. and Barcelo, D. (2008). Fully automated determination in the low nanogram per liter level of different classes of drugs of abuse in sewage water by on-line solid-phase extraction-liquid chromatography-electrospray-tandem mass spectrometry. *Analytical Chemistry* 80(9): 3123-3134.

Rehm, J., Baliunas, D., Brochu, S., Fischer, B., Gnam, W., Patra, J., et al. (2006). The Costs of Substance Abuse in Canada 2002: Highlights.

Ricaurte, G. A., McCann, U. D., Szabo, Z. and Scheffel, U. (2000). Toxicodynamics and long-term toxicity of the recreational drug, 3, 4-methylenedioxymethamphetamine (MDMA, 'Ecstasy'). *Toxicology Letters* 112-113: 143-146.

Rothman, R. B., Baumann, M. H., Dersch, C. M., Romero, D. V., Rice, K. C., Carroll, F. I., et al. (2001). Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse* 39(1): 32-41.

Saxena, S. and Donoghoe, M. C. (2000). Introduction. *Guide to Drug Abuse Epidemiology*. Geneva, World Health Organization.

Schep, L. J., Slaughter, R. J. and Beasley, D. M. (2010). The clinical toxicology of metamfetamine. *Clinical Toxicology (Philadelphia, Pa.)* 48(7): 675-694.

Schifano, F., Albanese, A., Fergus, S., Stair, J. L., Deluca, P., Corazza, O., et al. (2011). Mephedrone (4-methylmethcathinone; 'meow meow'): chemical, pharmacological and clinical issues. *Psychopharmacology (Berl)* 214(3): 593-602.

Shaw, K. P. (1999). Human methamphetamine-related fatalities in Taiwan during 1991-1996. *Journal of Forensic Sciences* 44(1): 27-31.

Shimosato, K. (1988). Urinary excretion of p-hydroxylated methamphetamine metabolites in man. II. Effect of alcohol intake on methamphetamine metabolism. *Pharmacology Biochemistry and Behavior* 29(4): 733-740.

Shirk, D. A. (2011). The drug war in Mexico: confronting a shared threat. New York, Council on Foreign Relations.

Siegel, R. K. (1977). Recreational Use and Intoxication. *NIDA Research Monograph*. Series 13: 119-136.

Simpson, L. L. (1975). Blood pressure and heart rate responses evoked by d- and l-amphetamine in the pithed rat preparation. *Journal of Pharmacology and Experimental Therapeutics* 193(1): 149-159.

Smart, R. G. (2000). Reporting Systems. *Guide to Drug Abuse Epidemiology*. World Health Organization. Geneva, World Health Organization.

Staack, R. F., Fritschi, G. and Maurer, H. H. (2002). Studies on the metabolism and toxicological detection of the new designer drug N-benzylpiperazine in urine using gas chromatography-mass spectrometry. *Journal of Chromatography B* 773(1): 35-46.

Staack, R. F., Fritschi, G. and Maurer, H. H. (2003). New designer drug 1-(3-trifluoromethylphenyl) piperazine (TFMPP): gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry studies on its phase I and II metabolism and on its toxicological detection in rat urine. *Journal of Mass Spectrometry* 38(9): 971-981.

Staack, R. F. and Maurer, H. H. (2005). Metabolism of designer drugs of abuse. *Current Drug Metabolism* 6(3): 259-274.

Staack, R. F., Paul, L. D., Springer, D., Kraemer, T. and Maurer, H. H. (2004). Cytochrome P450 dependent metabolism of the new designer drug

1-(3-trifluoromethylphenyl)piperazine (TFMPP). In vivo studies in Wistar and Dark Agouti rats as well as in vitro studies in human liver microsomes. *Biochemical Pharmacology* 67(2): 235-244.

Stafford, J. (2009). What are the IDRS and EDRS and what do they tell us? Sydney, National Drug and Alcohol Research Centre.

Strano-Rossi, S., Cadwallader, A. B., de la Torre, X. and Botre, F. (2010). Toxicological determination and in vitro metabolism of the designer drug methylenedioxypyrovalerone (MDPV) by gas chromatography/mass spectrometry and liquid chromatography/quadrupole time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry* 24(18): 2706-2714.

Substance Abuse and Mental Health Services Administration (2012). Results from the 2010 National Survey on Drug Use and Health: Detailed Tables. Rockville.

Sulzer, D., Sonders, M. S., Poulsen, N. W. and Galli, A. (2005). Mechanisms of neurotransmitter release by amphetamines: a review. *Progress in Neurobiology* 75(6): 406-433.

Tarr, J. E. and Macklin, M. (1987). Cocaine. *Pediatric Clinics of North America* 34(2): 319-331.

Taylor, D. and Ho, B. T. (1978). Comparison of inhibition of monoamine uptake by cocaine, methylphenidate and amphetamine. *Research Communications in Chemical Pathology and Pharmacology* 21(1): 67-75.

Terzic, S., Senta, I. and Ahel, M. (2010). Illicit drugs in wastewater of the city of Zagreb (Croatia) - Estimation of drug abuse in a transition country. *Environmental Pollution* 158(8): 2686-2693.

Thompson, I., Williams, G., Sarah Aldington, Williams, M., Caldwell, B., Dickson, S., et al. (2006). The benzylpiperazine (BZP) / trifluoromethylphenylpiperazine (TFMPP) and

alcohol safety study. Wellington, Medical Research Institute of New Zealand.

Topp, L., Breen, C., Kaye, S. and Darke, S. (2004). Adapting the Illicit Drug Reporting System (IDRS) to examine the feasibility of monitoring trends in the markets for 'party drugs'. *Drug and Alcohol Dependence* 73(2): 189-197.

Tsutsumi, H., Katagi, M., Miki, A., Shima, N., Kamata, T., Nishikawa, M., et al. (2005). Development of simultaneous gas chromatography-mass spectrometric and liquid chromatography-electrospray ionization mass spectrometric determination method for the new designer drugs, N-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)piperazine (TFMPP) and their main metabolites in urine. *Journal of Chromatography B* 819(2): 315-322.

United Nations Office on Drugs and Crime (2005). World Drug Report 2005. Volume 1: Analysis. New York, UNODC.

United Nations Office on Drugs and Crime (2010). Global Smart Update. 3.

United Nations Office on Drugs and Crime (UNODC) (2008). World Drug Report 2008. Vienna.

United Nations Office on Drugs and Crime (UNODC) (2009). World Drug Report 2009. New York, UNODC.

United Nations Office on Drugs and Crime (UNODC) (2010). World Drug Report 2010. New York, UNODC.

United Nations Office on Drugs and Crime (UNODC) (2011). World Drug Report 2011. New York, UNODC.

United Nations Office on Drugs and Crime (UNODC) (2012). World Drug Report 2012. New York, UNODC.

United States Department of Justice and National Drug Intelligence Center (2011). The

Economic Impact of Illicit Drug Use on American Society. Washinton, D. C.

Van Dyke, C., Jatlow, P., Ungerer, J., Barash, P. G. and Byck, R. (1978). Oral cocaine: plasma concentrations and central effects. *Science* 200(4338): 211-213.

van Nuijs, A. L., Castiglioni, S., Tarcomnicu, I., Postigo, C., de Alda, M. L., Neels, H., et al. (2011). Illicit drug consumption estimations derived from wastewater analysis: a critical review. *Science of the Total Environment* 409(19): 3564-3577.

van Nuijs, A. L., Gheorghe, A., Jorens, P. G., Maudens, K., Neels, H. and Covaci, A. (2013). Optimization, validation, and the application of liquid chromatography-tandem mass spectrometry for the analysis of new drugs of abuse in wastewater. *Drug Testing and Analysis*.

van Nuijs, A. L., Mougel, J. F., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P. G., et al. (2011). Sewage epidemiology--a real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium. *Environment International* 37(3): 612-621.

van Nuijs, A. L., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P. G., et al. (2009a). Can cocaine use be evaluated through analysis of wastewater? A nation-wide approach conducted in Belgium. *Addiction* 104(5): 734-741.

van Nuijs, A. L., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P. G., et al. (2009b). Cocaine and metabolites in waste and surface water across Belgium. *Environmental Pollution* 157(1): 123-129.

van Nuijs, A. L., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P. G., et al. (2009c). Spatial and temporal variations in the occurrence of cocaine and benzoylecgonine in waste- and surface water from Belgium and removal during wastewater treatment. *Water Research* 43(5): 1341-1349.

van Nuijs, A. L., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P. G., Neels, H., et al. (2009). Analysis of drugs of abuse in wastewater by hydrophilic interaction liquid

chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* 395(3): 819-828.

van Nuijs, A. L. N., Abdellati, K., Bervoets, L., Blust, R., Jorens, P. G., Neels, H., et al. (2012). The stability of illicit drugs and metabolites in wastewater, an important issue for sewage epidemiology? *Journal of Hazardous Materials*.

Verebey, K., Alrazi, J. and Jaffe, J. H. (1988). The complications of 'ecstasy' (MDMA). *The Journal of the American Medical Association* 259(11): 1649-1650.

White, N., Vial, R. and Ali, R. (2008). SA Drug Trends 2007. Findings from the Illicit Drug Reporting System (IDRS), Drug and Alcohol Services South Australia.

White, R. H. and Standen, O. D. (1953). Piperazine in the treatment of threadworms in children; report on a clinical trial. *British Medical Journal* 2(4839): 755-757.

Winstock, A. R., Mitcheson, L. R., Deluca, P., Davey, Z., Corazza, O. and Schifano, F. (2011). Mephedrone, new kid for the chop? *Addiction* 106(1): 154-161.

Wolff, K., Tsapakis, E. M., Winstock, A. R., Hartley, D., Holt, D., Forsling, M. L., et al. (2006). Vasopressin and oxytocin secretion in response to the consumption of ecstasy in a clubbing population. *Journal of Psychopharmacology* 20(3): 400-410.

Wood, D. M. and Dargan, P. I. (2012). Understanding how data triangulation identifies acute toxicity of novel psychoactive drugs. *Journal of Medical Toxicology* 8(3): 300-303.

World Health Organization (2002). The World Health Report 2002. Genova.

World Health Organization (2010). ATLAS on substance use (2010): Resources for the prevention and treatment of substance use disorders. Geneva.

Zhu, B. L., Oritani, S., Shimotouge, K., Ishida, K., Quan, L., Fujita, M. Q., et al. (2000). Methamphetamine-related fatalities in forensic autopsy during 5 years in the southern half of Osaka city and surrounding areas. *Forensic Science International* 113(1-3): 443-447.

Zuba, D. and Byrska, B. (2012). Prevalence and co-existence of active components of 'legal highs'. *Drug Testing and Analysis*.

Zuccato, E., Castiglioni, S., Bagnati, R., Chiabrando, C., Grassi, P. and Fanelli, R. (2008). Illicit drugs, a novel group of environmental contaminants. *Water Research* 42(4-5): 961-968.

Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R. and Fanelli, R. (2008). Estimating community drug abuse by wastewater analysis. *Environmental Health Perspectives* 116(8): 1027-1032.

Zuccato, E., Chiabrando, C., Castiglioni, S., Calamari, D., Bagnati, R., Schiarea, S., et al. (2005). Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. *Environmental Health* 4: 14.