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Assessing the added value of wastewater-based epidemiology to monitor illicit drug use

Béen Frederic

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Assessing the added value of wastewater-based
epidemiology to monitor illicit drug use

PhD Thesis

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IMPRIMATUR

A l'issue de la soutenance de thèse, le Jury autorise l'impression de la thèse de M. Frederic Béen, candidat au doctorat en science forensique, intitulée

« Assessing the added value of wastewater-based epidemiology to monitor illicit drug use »

La Présidente du Jury


Professeure Manon Jendly

Lausanne, le 11 décembre 2015

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Abstract

Wastewater-based epidemiology consists in acquiring relevant information about the lifestyle and health status of the population through the analysis of wastewater samples collected at the influent of a wastewater treatment plant. Whilst being a very young discipline, it has experienced an astonishing development since its first application in 2005. The possibility to gather community-wide information about drug use has been among the major field of application. The wide resonance of the first results sparked the interest of scientists from various disciplines. Since then, research has broadened in innumerable directions. Although being praised as a revolutionary approach, there was a need to critically assess its added value, with regard to the existing indicators used to monitor illicit drug use.

The main, and explicit, objective of this research was to evaluate the added value of wastewater-based epidemiology with regards to two particular, although interconnected, dimensions of illicit drug use. The first is related to trying to understand the added value of the discipline from an epidemiological, or societal, perspective. In other terms, to evaluate if and how it completes our current vision about the extent of illicit drug use at the population level, and if it can guide the planning of future prevention measures and drug policies. The second dimension is the criminal one, with a particular focus on the networks which develop around the large demand in illicit drugs. The goal here was to assess if wastewater-based epidemiology, combined to indicators stemming from the epidemiological dimension, could provide additional clues about the structure of drug distribution networks and the size of their market.

This research had also an implicit objective, which focused on initiating the path of wastewater-based epidemiology at the *Ecole des Sciences Criminelles* of the University of Lausanne. This consisted in gathering the necessary knowledge about the collection, preparation, and analysis of wastewater samples and, most importantly, to understand how to interpret the acquired data and produce useful information.

In the first phase of this research, it was possible to determine that ammonium loads, measured directly in the wastewater stream, could be used to monitor the dynamics of the population served by the wastewater treatment plant. Furthermore, it was shown that on the long term, the population did not have a substantial impact on consumption patterns measured through wastewater analysis.

Focussing on methadone, for which precise prescription data was available, it was possible to show that reliable consumption estimates could be obtained via wastewater analysis. This allowed to validate the selected sampling strategy, which was then used to monitor the consumption of heroin, through the measurement of morphine. The latter, in combination to prescription and sales data, provided estimates of heroin consumption in line with other indicators. These results, combined to epidemiological data, highlighted the good

correspondence between measurements and expectations and, furthermore, suggested that the dark figure of heroin users evading harm-reduction programs, which would thus not be measured by conventional indicators, is likely limited.

In the third part, which consisted in a collaborative study aiming at extensively investigating geographical differences in drug use, wastewater analysis was shown to be a useful complement to existing indicators. In particular for stigmatised drugs, such as cocaine and heroin, it allowed to decipher the complex picture derived from surveys and crime statistics. Globally, it provided relevant information to better understand the drug market, both from an epidemiological and repressive perspective.

The fourth part focused on cannabis and on the potential of combining wastewater and survey data to overcome some of their respective limitations. Using a hierarchical inference model, it was possible to refine current estimates of cannabis prevalence in the metropolitan area of Lausanne. Wastewater results suggested that the actual prevalence is substantially higher compared to existing figures, thus supporting the common belief that surveys tend to underestimate cannabis use. Whilst being affected by several biases, the information collected through surveys allowed to overcome some of the limitations linked to the analysis of cannabis markers in wastewater (i.e., stability and limited excretion data). These findings highlighted the importance and utility of combining wastewater-based epidemiology to existing indicators about drug use.

Similarly, the fifth part of the research was centred on assessing the potential uses of wastewater-based epidemiology from a law enforcement perspective. Through three concrete examples, it was shown that results from wastewater analysis can be used to produce highly relevant intelligence, allowing drug enforcement to assess the structure and operations of drug distribution networks and, ultimately, guide their decisions at the tactical and/or operational level.

Finally, the potential to implement wastewater-based epidemiology to monitor the use of harmful, prohibited and counterfeit pharmaceuticals was illustrated through the analysis of sibutramine, and its urinary metabolite, in wastewater samples.

The results of this research have highlighted that wastewater-based epidemiology is a useful and powerful approach with numerous scopes. Faced with the complexity of measuring a hidden phenomenon like illicit drug use, it is a major addition to the panoply of existing indicators.

Résumé

L'épidémiologie basée sur l'analyse des eaux usées (ou, selon sa définition anglaise, « wastewater-based epidemiology ») consiste en l'acquisition d'informations portant sur le mode de vie et l'état de santé d'une population via l'analyse d'échantillons d'eaux usées récoltés à l'entrée des stations d'épuration. Bien qu'il s'agisse d'une discipline récente, elle a vécu des développements importants depuis sa première mise en œuvre en 2005, notamment dans le domaine de l'analyse des résidus de stupéfiants. Suite aux retombées médiatiques des premiers résultats de ces analyses de métabolites dans les eaux usées, de nombreux scientifiques provenant de différentes disciplines ont rejoint les rangs de cette nouvelle discipline en développant plusieurs axes de recherche distincts. Bien que reconnu pour son côté objectif et révolutionnaire, il était nécessaire d'évaluer sa valeur ajoutée en regard des indicateurs couramment utilisés pour mesurer la consommation de stupéfiants.

En se focalisant sur deux dimensions spécifiques de la consommation de stupéfiants, l'objectif principal de cette recherche était focalisé sur l'évaluation de la valeur ajoutée de l'épidémiologie basée sur l'analyse des eaux usées. La première dimension abordée était celle épidémiologique ou sociétale. En d'autres termes, il s'agissait de comprendre si et comment l'analyse des eaux usées permettait de compléter la vision actuelle sur la problématique, ainsi que déterminer son utilité dans la planification des mesures préventives et des politiques en matière de stupéfiants actuelles et futures. La seconde dimension abordée était celle criminelle, en particulier, l'étude des réseaux qui se développent autour du trafic de produits stupéfiants. L'objectif était de déterminer si cette nouvelle approche combinée aux indicateurs conventionnels, fournissait de nouveaux indices quant à la structure et l'organisation des réseaux de distribution ainsi que sur les dimensions du marché.

Cette recherche avait aussi un objectif implicite, développer et évaluer la mise en place de l'épidémiologie basée sur l'analyse des eaux usées. En particulier, il s'agissait d'acquérir les connaissances nécessaires quant à la manière de collecter, traiter et analyser des échantillons d'eaux usées, mais surtout, de comprendre comment interpréter les données afin d'en extraire les informations les plus pertinentes.

Dans la première phase de cette recherche, il a pu être mis en évidence que les charges en ammonium, mesurées directement dans les eaux usées permettait de suivre la dynamique des mouvements de la population contributrice aux eaux usées de la station d'épuration de la zone étudiée. De plus, il a pu être démontré que, sur le long terme, les mouvements de la population n'avaient pas d'influence substantielle sur le pattern de consommation mesuré dans les eaux usées.

En se focalisant sur la méthadone, une substance pour laquelle des données précises sur le nombre de prescriptions étaient disponibles, il a pu être démontré que des estimations exactes sur la consommation pouvaient être tirées de l'analyse des eaux usées. Ceci a permis

de valider la stratégie d'échantillonnage adoptée, qui, par le biais de la morphine, a ensuite été utilisée pour suivre la consommation d'héroïne. Combinée aux données de vente et de prescription, l'analyse de la morphine a permis d'obtenir des estimations sur la consommation d'héroïne en accord avec des indicateurs conventionnels. Ces résultats, combinés aux données épidémiologiques ont permis de montrer une bonne adéquation entre les projections des deux approches et ainsi démontrer que le chiffre noir des consommateurs qui échappent aux mesures de réduction de risque, et qui ne seraient donc pas mesurés par ces indicateurs, est vraisemblablement limité.

La troisième partie du travail a été réalisée dans le cadre d'une étude collaborative qui avait pour but d'investiguer la valeur ajoutée de l'analyse des eaux usées à mettre en évidence des différences géographiques dans la consommation de stupéfiants. En particulier pour des substances stigmatisées, telles la cocaïne et l'héroïne, l'approche a permis d'objectiver et de préciser la vision obtenue avec les indicateurs traditionnels du type sondages ou les statistiques policières. Globalement, l'analyse des eaux usées s'est montrée être un outil très utile pour mieux comprendre le marché des stupéfiants, à la fois sous l'angle épidémiologique et répressif.

La quatrième partie du travail était focalisée sur la problématique du cannabis ainsi que sur le potentiel de combiner l'analyse des eaux usées aux données de sondage afin de surmonter, en partie, leurs limitations. En utilisant un modèle d'inférence hiérarchique, il a été possible d'affiner les actuelles estimations sur la prévalence de l'utilisation de cannabis dans la zone métropolitaine de la ville de Lausanne. Les résultats ont démontré que celle-ci est plus haute que ce que l'on s'attendait, confirmant ainsi l'hypothèse que les sondages ont tendance à sous-estimer la consommation de cannabis. Bien que biaisés, les données récoltées par les sondages ont permis de surmonter certaines des limitations liées à l'analyse des marqueurs du cannabis dans les eaux usées (i.e., stabilité et manque de données sur l'excrétion). Ces résultats mettent en évidence l'importance et l'utilité de combiner les résultats de l'analyse des eaux usées aux indicateurs existants.

De la même façon, la cinquième partie du travail était centrée sur l'apport de l'analyse des eaux usées du point de vue de la police. Au travers de trois exemples, l'utilisation de l'indicateur pour produire du renseignement concernant la structure et les activités des réseaux de distribution de stupéfiants, ainsi que pour guider les choix stratégiques et opérationnels de la police, a été mise en évidence.

Dans la dernière partie, la possibilité d'utiliser cette approche pour suivre la consommation de produits pharmaceutiques dangereux, interdits ou contrefaits, a été démontrée par l'analyse dans les eaux usées de la sibutramine et ses métabolites.

Les résultats de cette recherche ont mis en évidence que l'épidémiologie par l'analyse des eaux usées est une approche pertinente et puissante, ayant de nombreux domaines

d'application. Face à la complexité de mesurer un phénomène caché comme la consommation de stupéfiants, la valeur ajoutée de cette approche a ainsi pu être démontrée.

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Abbreviations

- 5-HIAA: 5-hydroxyindoleacetic acid
- 6-MAM: 6-monoacetylmorphine
- BE: Benzoyllecgonine
- BOD: Biochemical oxygen demand
- COC: Cocaine
- COD: Chemical oxygen demand
- EDDP: 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
- EMCDDA: European Monitoring Centre for Drugs and Drug Addiction
- EPFL: Ecole Polytechnique Fédérale de Lausanne
- GC: Gas chromatography
- GPS: General population survey
- HMMA: 4-hydroxy-3-methoxymethamphetamine
- HRMS: High-resolution mass spectrometry
- IDU: Injecting drug user
- INCB: International Narcotics Control Board
- ISE: Ion-selective electrode
- LC: Liquid chromatography
- LVI: Large-volume injection
- MCMC: Markov Chain Monte Carlo
- MDA: (±)-3,4-Methylenedioxyamphetamine
- MDEA: (±)-3,4-Methylenedioxyethylamphetamine
- MDMA: (±)-3,4-Methylenedioxymethamphetamine
- MRM: Multiple-reaction-monitoring
- MS: Mass spectrometry
- MS/MS: Tandem mass spectrometry
- N: Nitrogen
- NH₄-N: Ammonium linked nitrogen measured in wastewater
- NPS: New psychoactive substances
- OH-THC: 11-Hydroxy- Δ^9 -tetrahydrocannabinol
- P: Phosphorous
- PE: Population equivalent
- PPCPs: Pharmaceuticals and personal care products
- SBE: Sewage-based epidemiology
- SPE: Solid-phase extraction

-
- Swissmedic: Swiss Agency for Therapeutic Drugs
 - THC-COOH: 11-nor-9-carboxy-delta-9-tetrahydrocannabinol
 - UNODC: United Nations Office for Drugs and Crime
 - WBE: Wastewater-based epidemiology
 - WWTP: Wastewater treatment plant

I Introduction

Consumption of drugs of abuse is one of the major concerns in human health worldwide. According to the latest estimates provided by the United Nations Office on Drugs and Crime (UNODC), approximately 246 million people, aged 15 or older, used an illicit drug in 2013 (1). But most importantly, one out of ten, in other words almost 27 million people worldwide, are considered to be problem drug users (1). This particular group of users, on which the harmful effects of drug use will be the most serious, will also lead to the highest human and economical costs for society. These figures emphasise the need to continuously develop new strategies, rethink drug policies as well as prevention and treatment programs. Yet, this can only be achieved by having at hand data which allows to monitor illicit drug use at the population level, detect changing trends in types of drugs being used and identify new substances (e.g., new psychoactive substances, NPS (2)). Strategies available to measure drug use are mainly based on the combination of various epidemiological, sociological and criminological tools (e.g., population surveys, crime statistics) (3). Nevertheless, these classical methods present a certain number of limitations linked to the validity of the obtained results, which cannot be overcome. Taking these into account, the UNODC highlighted the importance of implementing sound and harmonised methods to produce robust statistics about illicit drug use (4). Furthermore, in light of current movements to revise present drug policies, the need to adopt alternative, scientific and evidence-based methods for “[...] monitoring and measuring production, trafficking and consumption” has been stressed (p. 1 (5)). It is in this context of urgent need to develop new indicators, aimed at refining current knowledge about drug use, that wastewater analysis finds its place.

Drug use, however, has another equally important dimension that should not be neglect, namely the criminal one. In fact, the large demand in illicit drugs is a flourishing environment for criminal groups making exorbitant profits. Yet, their scheme seldom limit to drugs and often involves also other criminal activities such as weapon and human trafficking. At present, accurately defining the size of the market is a difficult task. Thus, one can only hardly define the context in which drug-related criminal activities should be assessed. This holds true for measures taken by authorities to counter these, whose concrete effect cannot be evaluated. Wastewater analysis could prove a useful tool to put a figure on size of the market and thus improve current understanding about how criminal networks are structured and how they operate.

In 2007, an international meeting was organized to discuss the possibility of applying this innovative method to measure illicit drug use at the population level (6). Since this meeting, an extraordinary number of studies, focusing on the refinement and the application of wastewater analysis, have been published in the scientific literature. In the context of this work, the contribution of wastewater to the understanding of the two dimensions of illicit drug use, illustrated in Figure 1, will be evaluated.

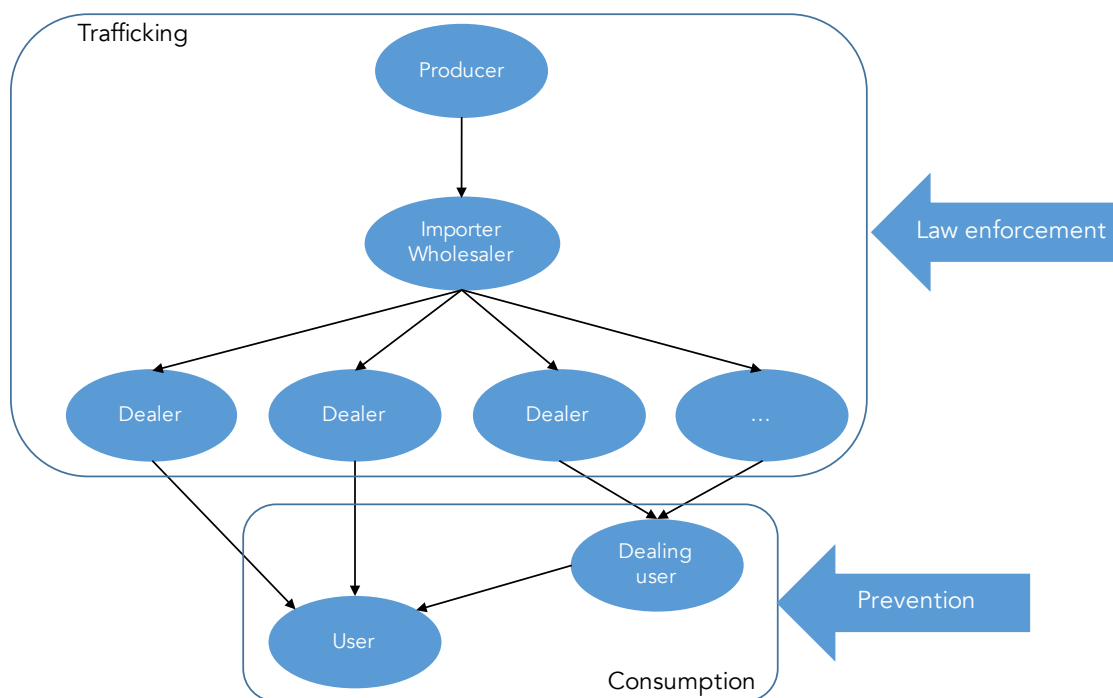


Figure 1: Illustration of the two dimensions of illicit drug use, *Trafficking* and *Consumption*, which will be the focus of this work.

1.1 Current methods to monitor illicit drug use

One of the key parameters that addiction researchers and epidemiologists seek to measure is the prevalence of illicit drug use in the general population. This is particularly important from an epidemiological perspective, as it will allow to assess the consequences that consumption of a certain substance will have on the population, in terms of health but also crime levels. However, as will be discussed in the following section, the tangible consequences that drug use has on society can be measured through a variety of other indicators, all of which are extremely useful to addiction researchers as they allow to better understand the phenomenon as a whole.

Indicators used to measure illicit drug use can be separated into direct and indirect methods. The literature in the domain provides an exhaustive explanation of the various approaches and their limitations. Detailing them all goes beyond the scope of this work. Thus, only a synthetic overview will be presented here.

1.1.1 Direct methods: Population surveys

Direct methods are mainly based on general population surveys, which are probabilistic surveys carried out among a given population, represent one of the major key indicators, used for instance by the European Monitoring Centre for Drug and Drug Addictions (EMCDDA) (7). This type of data source is widely used to estimate the extent of illicit drug consumption and provides insights into patterns of use. At the national level, general population survey are helpful for monitoring purposes, to identify major problems as well as to plan (and evaluate)

responses and drug policies (8). One of the major advantages of this indicator is that it provides detailed information about the consumption habits (e.g., life, yearly or monthly prevalence), risk perception, social and health status as well as the consequence of drug abuse among individuals. This information is gathered for each questioned person and can then be used to investigate relationships between drug abuse/consumption patterns and other variables (e.g., gender, age, years since first injection, number and type of treatments followed). In Switzerland, a telephonic survey aimed at gathering information about drug use in the general population has been carried out yearly since 2011 (9). Other surveys, whose goal is to collect information about criminal behaviour in the broader sense (including drug use) (10) or surveys targeting specific subgroups of the population (e.g., nightlife and partygoers (11)) have also been carried out. Particular types of population surveys are those conducted among students (mainly between 11 and 15 years old). Two important surveys, which are carried out on a regular base, are the European School Survey Project on Alcohol and other Drugs (ESPAD) (12) and the Health Behaviour in School Children (HBSC) (13). In Switzerland, this type of study was last carried out in 2014 among school children (aged 11-15) (14).

However, as every type of measurement tool, population surveys present some limitations. Firstly, there is a problem in reliability since the sample size is reduced and represents only a small fraction of the entire population. For example, in the case of school children surveys, these focus only on a age-specific cohort, thus making it difficult to extrapolate information relative to the entire population. Yet, one can draw information about the status of future generations. Secondly, even by considering a large sample, there will be biases due to concealment (caused by the negative vision associated to illicit drug abuse), subjective interpretation and memory biases. Thirdly, they are not adequate for monitoring the consumption among problem drug users (i.e., individuals who have recourse to drug injection or are long-term/regular users of opioids, cocaine and/or amphetamines) as these persons tend to be marginalized and are difficult to include among the questioned people (3,8). Nevertheless, some of these limitations are less severe when considering substances which prevalence is higher (mainly cannabis). In the specific case of schoolchildren surveys, particular attention has to be paid as the questioning occurs among a very sensitive age group. Finally, the number of general population surveys that are carried out is limited because they are expensive and time consuming (performed every 2 to 4 years in the best cases). Thus, the time laps between collection and data analysis/interpretation can be extremely long, making it almost impossible to produce real-time data about illicit drug consumption. In fact, data is generally reported as life prevalence or consumption frequency in the year/month prior to questioning.

1.1.2 Indirect methods

Because monitoring the extent of drug use based solely on direct methods is not enough to provide unbiased data, so called indirect methods have been developed. These extrapolate drug-related information from other statistical resources like police statistics about drug seizures, offences and criminal prosecutions (e.g., consumption and trafficking). Although

being influenced by current strategies, themselves affected by external factors such as public opinion and politics, data deriving from drug enforcement activities can, within its limits, be used to evaluate the drug market and, more importantly, to understand how criminal networks operate.

Beyond law enforcement and crime statistics, additional indicators are commonly used in complement of population surveys (7). i) Problem drug use indicator, which is used to monitor more problematic and specific drug use patterns that are not captured by general population surveys, such as heroin injection and/or long-term cocaine consumption (15). These often consists in questionnaires conducted among people visiting low threshold facilities (16). Because it focuses on a specific population, this kind of tool provides valuable information, but limited to a restraint group of often marginalized heavy drug users. ii) Drug-related deaths and mortality among drug users are also among the commonly used indicators (17). Two types of data sources are used to construct this indicator. The first, and direct source, are deaths directly associated to drug use which can be retrieved from national death statistics. The second consists in estimations based on investigation of mortality among specific cohorts (e.g., heroin users or, in the specific case of Switzerland, people under heroin or methadone treatment). In this case, not only deaths directly associated to drug use are included, but also indirect causes (e.g., accidents, suicides and violence) (17). This particular type of data is very interesting for determining long-term effects of drug use, identify risk-associated consumption patterns (e.g., poly drug use), detect (new) dangerous products, evaluate the efficiency of treatment programs (when cohort studies are being considered) and drug policies. Still, this indicator suffers from limitations due to the absence of complete investigation in cases of unnatural or violent deaths (although less likely in developed countries, where thorough investigations are carried out in case of violent deaths), it is subject to classification/categorization biases, it requires a good cooperation and commitment between the various institutions and, last but not least, it mainly provides information about high-risk substances (e.g., heroin) (17). iii) Treatment demands provide information about the number of demands, the status of people seeking treatment (e.g., age, gender, social/economical situation, prevalence and consumption habits), trends among problem drug users and allows planning and evaluating the effectiveness of treatment facilities (18). In Switzerland, this type of statistics are compiled on a yearly basis (19). iv) Statistics about drug-related infectious diseases (mainly HIV, Hepatitis A and B), are mainly used to monitor the levels of injection and their trends over time among injecting drug users (20). It provides useful information for planning prevention campaigns and treatment facilities, and it indirectly allows to estimate incidence and prevalence of drug injection. Analogously to other cohort-specific indicators, its' results are specific only of that group. Even if these various types of indicators gather very useful information about illicit drug consumption, they are not able to provide the entire picture. In particular, those users whose behaviour is not severe will not be considered, as they are less likely to be included in statistics who focus mainly on problem drug users (3). Furthermore, data reporting in Europe and the rest of the world is particularly variable and there is still no standardized method to collect information relative to drug consumption in Europe (21).

Analogously to population surveys, indirect methods are often not capable of providing a rapid picture of the situation and their use in longitudinal studies is very demanding.

1.2 Objectives of the research

Illicit drug use has been shown to be a global problem, whose consequences touch health, societal and juridical aspects. Yet, because of its numerous facets, it is an extremely complex phenomenon to understand and measure. Current knowledge about how drug use originates, develops and affects, directly or indirectly, people's life and societies is limited by the flaws of methods used to measure it. It is in this context that the analysis, in wastewater, of markers of drug use has seen the light. Presented as an objective, evidence-based and non-invasive approach, it has seen great developments in the past decade. However, as with every approach trying to model a complex phenomena, no matter how sophisticate, it will always only provide a partial description of it.

The overall goal of the present research was to investigate the added value of wastewater analysis as indicator of illicit drug consumption at the community level. In particular, to evaluate its potential to deliver new and useful insights into this complex phenomenon, from the perspective of public health and law enforcement. More in detail, the following objectives were accomplished:

Chapter III: Develop a population marker and evaluate how population dynamics affect patterns of drug use measured in wastewater.

This part of the work was published: Been F., Rossi L., Ort C., Rudaz S., Delémont O., Esseiva P., *Population Normalization with Ammonium in Wastewater-Based Epidemiology: Application to Illicit Drug Monitoring* Environmental Science & Technology, 2014, 48 (14), 8162–69; <http://dx.doi.org/10.1021/es5008388>

Chapter IV: Validate the methodology, with a particular focus on the selected sampling approach, by focusing on a specific substance for which detailed prescription data was available. Furthermore, a first attempt is made to combine results from wastewater analysis with existing epidemiological indicators.

This part of the work was published: Been F., Benaglia L., Lucia S., Gervasoni J-P., Esseiva P., Delémont O., *Data Triangulation in the Context of Opioid Monitoring via Wastewater Analyses*, Drug and Alcohol Dependence, 2015, 151, 203-210; <http://dx.doi.org/10.1016/j.drugalcdep.2015.03.022>

Chapter V: Investigate geographical differences in illicit drug use highlighted through wastewater analysis and their comparison with existing epidemiological and crime statistics.

This part of the work has been submitted for publication: Been F., Bijlsma L., Benaglia L., Berset J-D., Botero-Coy A. M., Castiglioni S., Kraus L., Zobel L., Schaub M., Bücheli A., Hernández F., Delémont O., Esseiva P., Ort C.; *Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland.* submitted.

Chapter VI: Develop an approach to refine current knowledge about prevalence of cannabis use, via the integration of wastewater and epidemiological data in a unique Bayesian hierarchical model.

This part of the work has been submitted for publication: Been F., Schneider C., Zobel F., Delémont O., Esseiva P.; *Integrating environmental and self-report data to refine cannabis prevalence estimates in a major urban area of Switzerland,* submitted.

Chapter VII: Evaluate the potential utility of wastewater analysis to improve the understanding of drug markets and trafficking, with a particular emphasis on the perspective of law enforcement.

Part of this work was published: Been F., Schneider C., Delémont O., Esseiva P.; *Können Abwasseranalysen der Polizei helfen, Drogenmärkte besser zu verstehen?*, *Kriminalistik*, 2015, 11, 628-634.

Chapter VIII: Evaluate potential extensions of wastewater-based epidemiology to other domains relevant to public health, with a particular focus on banned pharmaceuticals.

II Principles of wastewater-based epidemiology

In 2001, Daughton (22) conceived for the first time the possibility of monitoring drug use at the community level by detecting and quantifying these products and their metabolites in wastewaters. As the author stated in his paper, the approach is a “[...]rare bridge between the environmental and social sciences. [...] the use of non-intrusive drug monitoring at sewage treatment facilities and the use of the resulting non-incriminating data to determine collective drug usage parameters at the community levels [...]” (p. 1, (22)). The word “collective” plays here an important role. In fact, assessing consumption by means of analysing wastewater provides only information at the community level, since it is not possible to use such data to retrace the consumption patterns of a single individual. This simple but revolutionary idea resides on the assumption that all metabolites excreted after absorption of a xenobiotic product, and collected in sewer systems, are representative of the type and quantity of compound initially consumed. It would thus be possible to quantify these products and obtain useful information about substance use in the community served by the sewer system. This approach, which extends beyond illicit drugs but also includes pharmaceuticals, personal care products, health biomarkers, etc., has been referred to as wastewater-based epidemiology (WBE) or sewage-based epidemiology (SBE). When the focus is solely on illicit drugs, the approach has sometimes been referred to as wastewater-based drug epidemiology, although the term wastewater-based epidemiology will be used here.

This methodology was inspired by the already established practice of analysing waste and surface waters for assessing the presence of pharmaceuticals and personal care products (PPCPs). The environmental fate of PPCPs has been of concern in the scientific community since the beginning of the 1990's (23). The literature is now rich in studies which have investigated concentrations, impact and toxic effects of such products on the environment. This exponential growth in environmental toxicological studies has been promoted by the advances made in analytical chemistry. In particular, the coupling of chromatography and mass spectrometry and the development of efficient sample preparation techniques. These methods allowed scientists to detect and quantify substances in the sub-nanogram per litre range in very complex environmental matrices. As for pharmaceuticals, illicit drugs are likely to be ecotoxic due to their high pharmaceutical activity (24,25), yet until the beginning of 2000 these had never been looked for in the environment (26). Presence of illicit drugs in wastewaters was first reported in 2004 by Jones-Lepp and co-workers (27). Nevertheless, the first study whose focus was to implement the concept of monitoring drug use through analysis of environmental samples was carried out by Zuccato et al. and Castiglioni et al. (28,29) in 2005.

Since these first applications, numerous studies have been carried out, leading to the establishment of research groups (30,31). As will be discussed in the following sections, WBE has been applied at different scales: at a regional, national or international level, granting information about the consumption in large populations (here referred to as “open environment”); or, on the contrary, if the monitoring program aims specific premises (e.g.,

schools and penitentiary facilities) it could be employed to investigate the usage in specific communities (here referred to as “confined environment”).

Regardless of which environment is being investigated, this approach has, on the one hand, the potential to deliver valuable data which could help monitor illicit drug use, measure the effectiveness of prevention campaigns or drug policies and, if combined to existing epidemiological data, help achieving a better understanding of the phenomenon in public health perspective.

On the other hand, wastewater analysis has also the potential to deliver informative data from a forensic and judicial perspective. If longitudinal monitoring campaigns are set up and the collected data is thoroughly confronted to the results of drug enforcement activities, valuable pieces of information can be obtained. For example, the evolution of consumption and thus, indirectly, that of the drug market could be monitored and the obtained information could be used to define new strategies. The long-term effect of these could then be evaluated using wastewater analysis. Furthermore, results from the analysis of wastewater samples could be used as reference to define the drug market and evaluate the proportion seizures have with regards to its total size, as well as to put a figure on turnover and profits generated from trafficking.

2.1 Occurrence of illicit drug residues in wastewater

Fanelli summarised the underlying principle of WBE as follows: “Sewage waters represents the sum of what we ingest, use, metabolize, and excrete from our body, in an extreme diluted sample containing thousands of different compounds, each one carrying information about its nature, origin, and, therefore, potentially useful data on their significance at the population level” (p. 336, 32). In other words, WBE relies on the fact that any chemical uptake by humans will potentially be excreted via urine, faeces or other “secondary” excretion routes (e.g., sweat, saliva). Through domestic sewers, the proportion of these chemicals, which was not lost in the environment, will at some point reach a wastewater treatment plant (WWTP). Here, various treatment processes will take place to clean up wastewater before being discharged into the environment. Illicit drugs entering sewers through the aforementioned routes will be found as unchanged parent compounds as well as metabolites (formed in the body by spontaneous hydrolysis or by the action of different enzymes (33)). These substances are the cornerstone of WBE applications. Direct disposal (i.e., dumping) of illicit drugs in sewers, via toilets for example, has also been recognized as an alternative route, not directly linked to consumption, through which these substances could be found in wastewater samples.

2.1.1 Urine as source of drug residues in sewers

In toxicological and clinical analysis, urine has always been seen as the biological matrix of choice because of the facility of collection, but also because the involved metabolism mechanisms tend to transform xenobiotics in more hydrophilic compounds, which can then be eliminated via urine (34). Thus, compounds found in urine have generally been the focus of

WBE studies, although the importance of other excretion routes, in particular faeces, has been recently emphasized (35,36).

Composition and characteristics of urine in catchments, however, cannot be considered as being the same as those drawn from medical studies. Maurer et al. (37) suggest three reasons: i) the contribution from numerous individuals tends to average out its characteristics, ii) the non-sterile environment (e.g., microbial activity) are likely to modify its composition and iii) mixing with water tends to modify the ionic composition through the addition of elements like calcium and magnesium. As will be discussed further on, the combination of these factors can remarkably influence the stability of drug residues in wastewater.

The occurrence of urine in wastewater (i.e., its introduction into sewers via toilet flushes) is a stochastic process which has been the subject of some investigations because of its impact on the environment (38,39). In fact, it has been shown that water closets (WCs) are the most important wastewater generating domestic appliance (i.e., 30-50% of the total wastewater produced in England and Malta), and some interesting production patterns could be observed (39). Peaks were generally observed in the morning (one major peak between 6 and 9AM) and in the evening (two peaks between 7 and 11PM). Minima were observed during midday and late night, corresponding to periods where people are working or sleeping respectively. Additionally, evidence was found indicating that WCs are the major source of ammonia (NH₃), which is particularly interesting because it is a good marker of urine content. In fact, it is argued that approximately 80% of nitrogen in influents is due to urine and that most of the nitrogen content is due to ammonia (40). According to Butler et al. (39), 80-85% and 100% of the total ammonia content were due to WC flushes during the day and during the night, respectively. As will be discussed further on, the presence of ammonia, and in particular ammonium ion, is of interest for WBE applications.

There is a great inter-individual variability in volumes of urine produced, principally because of drinking and transpiration, although average urine volumes range from 200 to 400 mL per pulse and can be modelled using a gamma distribution (38). As already stated, the occurrence and amounts of urine play a great role in the planning and design of WWTP. One interesting aspect which has been raised by Rauch et al. (38), is the mobility of people, meaning that during the course of working days home toilets will be less used as people move to work. Thus, sanitary ware of these environments is more readily used, while the inverse may be true during evenings and weekends (38,41). Additionally, if one considers that people might have their homes or offices in locations served by different WWTP, further complexity is added to the modelling of urine contribution in a given WWTP.

2.1.2 Other biological matrices as sources of drug residues in sewers

Although being the most important and by far the most studied, urine is not the only excretion route by which illicit drugs and pharmaceuticals are excreted from the human body. Faeces, sweat and saliva are also sources of drug residues which could potentially contribute to concentrations measured in wastewater samples. The occurrence of illicit drugs in faeces is primarily due to the unabsorbed orally administered drugs or substances stemming from biliary excretions (34). For some substances, like delta-9-tetrahydrocannabinol (THC), this has

shown to be the major excretion route (42). Sweat and saliva have attracted the attention of toxicologists because, like urine, they can be easily collected and can potentially bear information about drug use (43). Illicit drugs occur in saliva mainly by passive diffusion through the lipid membrane separating from capillaries and tend to disappear within 12 to 24 hours (43). Similarly, illicit drugs and their metabolites occur in sweat through passive diffusion (43). Sweat stems from two types of glands which are present on the human body, i.e. eccrine and apocrine glands (44). The parameters governing the extent to which drugs are excreted through the skin depends on the pK_a and the diffusion of the uncharged form of the molecule through the glands cell wall (23,34). Excretion through sweat is promoted for those compounds that are neutral at pH values around 7.4 (23). Clearly, amounts of sweat produced also influence the amounts of drug residues found on one's skin (e.g., sport). Through washing and bathing, metabolites present on the surface of the skin might enter the sewer system and thus contribute to the measured loads. Although the impact of these alternative routes is likely negligible compared to urine and faeces, no experimental data exists to support this assumption (23).

2.1.3 Non-biological sources of drug residues in sewers

Another route by which illicit drugs could be found in wastewater, but also in the environment, is direct disposal. For unused or expired PPCPs, direct disposal can be a major source of pollution (23,45). However, in the case of illicit drugs there is a fundamental difference, as consumers do not have particular interests in getting rid of unused substances, except for some rare incidents when one tries to conceal consumption and/or possession to avoid criminal prosecution. Yet, residues of illicit drugs could also end up in sewers during and after consumption (e.g. remains of powder are cleaned off surfaces and flushed down the toilet). While these minor events are likely more difficult to detect when analysing wastewater samples, dumping of large amounts will notably increase the concentration of the parent compound and, eventually, of some metabolites, as these will be formed directly in the sewers. As such, they should be more easily recognised by the analyst. Nonetheless, to avoid this kind of situations, the use of exclusive and specific metabolites, which are absent or found only in negligible amounts in the bulk product, is favoured in WBE applications.

A final route which can be conceived and by which drug residues could be introduced in sewers is rain. Leftovers or dried urine could be washed away and, in combined sewers systems, where rainwater is collected and mixed to domestic and industrial wastewater, it could contribute to the measured concentrations. This route could become particularly important in connection with major public events, where the amount of leftovers or the proportion of individuals not urinating in toilets could become significantly high. Yet, in the latter case, it would still be pertinent to the monitoring of drug use as residues present in urine originate from consumption.

2.2 Pharmacokinetics of the major classes of illicit drugs

The founding principle of WBE is related to the mechanism by which the consumption of xenobiotics (e.g., illegal and prescription drugs) can be detected, and eventually quantified, by measuring the occurrence of the parent compound or its metabolites in biological matrices. Each xenobiotic will undergo specific pharmacokinetic and metabolization processes, which, depending on the substance, can last for longer periods and excretion products will thus be found in urine up to several days after administration. As stated by Zuccato et al. (46) the ideal drug marker should be “a major and exclusive excretion product (metabolite or unchanged parent drug) of the drug under study that is stable in wastewater” ((46), p. 1027). Furthermore, it should also show limited inter- and intra-individual variability and should be excreted at similar rates regardless of the administration route and the dose. Yet, none of the available drug residues fulfils all criteria. Firstly, pharmacokinetics and excretion rates depend on the individual, since body mass, diseases and other factors associated to the state of the consumer will influence the excretion rate for a given illicit drug (34). Secondly, excretion rates depend on the administration route, as substances will have different bioavailability depending on how they enter the organism. This is an important aspect with regard to pharmacokinetic studies available in the literature and commonly referenced in WBE approaches. In fact, many of these trials have been carried out using administration routes which do not reflect current user practices. For example, in one of the few studies where faecal excretion of metabolites of delta-9-tetrahydrocannabinol (THC), the major active compound found in cannabis, was investigated, THC was administered intravenously (47). Clearly, this does not correspond to practices expected from consumers, which will primarily smoke cannabis or, eventually, consume edibles containing THC. Another aspect which can influence excretion rates is the dose. In fact, for substances such as (±)-3,4-methylenedioxymethamphetamine (MDMA or ecstasy), the administered dose has been shown to influence the excretion rate (48–50). Finally, the generalisability of results obtained from studies reported in the literature can be challenged if one looks at the limited number of individuals tested. Clearly, this is linked to the ethical issues researchers face to when setting up such trails. Nonetheless results should be carefully evaluated if the number of individuals tested is limited.

For the most common drugs, quite an important number of studies have been carried out in the past four decades. Although, if taken individually, the results of some studies might be questionable because of one or more of the above reasons, more robust estimates of excretion can eventually be obtained if these are pooled together. Various approaches have been suggested in the literature to combine and summarise results from distinct studies, the most common of which is meta-analysis. The method consists in obtaining a weighted average of the parameter of interest based on the available studies (51).

As will be discussed in *Section 2.5.3*, the uncertainty in excretion rates can have a significant impact on the conclusions which can be drawn from wastewater analysis, in particular when these are used to retrospectively infer the amounts of substance initially consumed.

The following sections provide an overview of the metabolic pathways and excretion rates of the major classes of illicit drugs.

2.2.1 Cocainics

Cocaine has been the target of most WBE studies published in the literature, mainly because of its widespread use, in particular in Europe where most monitoring campaigns have been carried out (28,52–65). After consumption, between 85 and 90% of the initial cocaine dose is excreted in urines in various forms within 24h (66), as shown in Table 1. Liver carboxylases are responsible for the formation of benzoylecgonine, by the hydrolysis of the ester linkages of the parent compound. However, this reaction can also occur spontaneously at physiological pH (33,34). Cocaine, ecgoninemethylester and benzoylecgonine can then be further modified to form minor metabolites such as ecgonine, norcocaine and cocaethylene, the latter being excreted exclusively when cocaine and alcohol are coadministered (33,34). As mentioned previously, the administration route influences the metabolism of cocaine. For instance, after intranasal administration of 1.5mg per kg of body weight, cocaine and benzoylecgonine maximal concentrations were reached during the first hour and after 4-6 hours respectively (67), while a peak of benzoylecgonine was observed after 6-12h when a 25mg dose of cocaine was administered orally (33). This is likely due to the differences in bioavailability of the substance, which is more readily metabolised after intravenous injection.

Table 1: Summary of the metabolites and their corresponding excretion rates measured in urine after administration of cocaine by nasal insufflation.

<i>Major metabolites of interest</i>	<i>Urinary Excretion Rate [%]</i>	<i>Administration Route</i>	<i>References</i>
Cocaine	1-15	Nasal Insufflation	(33–35,68–71)
Benzoylecgonine	15-55		
Ecgoninemethylester	32-49		

2.2.2 Amphetamine-type stimulants

Amphetamine-type stimulants (ATS) represent a class of compounds derived from phenethylamine to which a methyl group is linked to the α carbon. Among the various molecules, the most encountered ones are (\pm)-Amphetamine, (\pm)-Methamphetamine, (\pm)-3,4-Methylenedioxymethamphetamine (MDMA), (\pm)-3,4-Methylenedioxyethylamphetamine (MDEA), (\pm)-3,4-Methylenedioxyamphetamine (MDA) and Ephedrine. However, with the ever growing number of NPS appearing on the market every year (72), this group of compounds has seen an important increase recent years. Because of the presence of a centre of symmetry, these compounds occur as enantiomers: R- (Levo- or (-)-amphetamine) and S-isomers (Dextro- or (+)-amphetamine), with the latter being pharmacologically more active compared to its enantiomer (34,73). Synthesis of ATS in illegal laboratories tends to produce racemic mixtures since criminals do not possess the knowledge or the instrumentation to obtain enantiopure drugs. This is not the case for industrial or pharmaceutical productions,

where products generally contain only one enantiomer or determined proportion of each of them.

The common ATS listed above, all share similar metabolism and excretion routes and amphetamine is often among the excreted metabolites, although some specificities exist depending on the administered ATS, the state of the user and its physiological conditions. In the case of more recent NPS, very little if none pharmacokinetic data is available. In particular urinary pH can have a dramatic influence on the excretion rates of the different metabolites. Acidic or basic conditions have been shown to influence greatly the excretion rates of amphetamine and methamphetamine. Low pH tends to increase the release of the unchanged parent compound, while the inverse is true for higher values (34,74). Table 2 summarizes the metabolites and expected excretion rates after administration of the major ATS.

The metabolic process involved in the excretion of MDA, MDMA, MDEA and the other derivatives have not yet been studied extensively. In the particular case of MDMA, an important variability in excretion rates of the parent compound have been attributed to its non-linear elimination, which increases as a function of the administered dose (48–50). In some WBE studies, an excretion rate of 65% of MDMA after consumption of the compound was used (46), yet an extensive review by Khan and Nicell (35) showed that this is not consistent with studies reported in the literature, and suggested an excretion rate for the unchanged parent compound between 12-47% of the initial dose. Specific metabolites of MDMA (i.e., 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA) (75)) have been reported in the literature and can be used as markers of MDMA consumption in WBE studies (76).

Table 2: Summary of the metabolites and their corresponding excretion rates measured in urine after administration of the major ATS. a) Average excretion rate observed in normal pH conditions.

<i>Administered compound</i>	<i>Major metabolites of interest</i>	<i>Urinary Excretion Rate [%]</i>	<i>Administration Route</i>	<i>References</i>
Amphetamine	AM	1-70 (30) ^a	Oral	(34,74)
Methamphetamine	METH	2-76 (43) ^a	Oral	(33,34,74)
	AM	7		
MDMA	MDMA	12-47	Oral	(48,77–79)
	HMMA	11-49		

Excretion of amphetamine and methamphetamine after administration of pharmaceuticals has been reported. In particular, amphetamine is excreted after consumption of phenethylamine (stimulating drug) and fenproporex (stimulating drug used to treat obesity), while methamphetamine is found after administration of selegeline (used to treat Parkinson's disease) and famprofazone (non-steroidal anti-inflammatory agent, NSAID) (33,52,80). Phenethylamine and fenproporex are submitted to strict control (classed as narcotic and/or psychotropic substances, type *a* and *b* products) (81) and are not listed among the commercialized pharmaceutical products in Switzerland (82). Selegeline and famprofazone are not mentioned among the pharmaceutical products listed by the Swiss Agency for Therapeutic Drugs (Swissmedic), neither among the commercialized products (81,82). So

there is no contribution expected from pharmaceuticals to quantities of amphetamine and methamphetamine measured in wastewater samples in collected in Switzerland.

2.2.3 Opioids

The term opioid represents the class of natural, semi-synthetic or synthetic alkaloid compounds prepared from opium. The subgroup of compounds naturally present in opium poppies, referred to as “opiates”, consists of morphine and codeine. Other semi-synthetic or synthetic active compounds derived from opium are heroin, oxycodone, buprenorphine, fentanyl, methadone, and tramadol, which all share similar pharmacokinetic characteristics (34). In sewage epidemiological studies, the mainly analysed compounds were morphine, heroin, codeine, methadone and their metabolites (52,53,56,83–85). Table 3 reports the excretion rates for the major opioids.

Table 3: Summary of the metabolites and their corresponding excretion rates measured in urine after administration of the major opioids. a) Morphine-3-glucuronide (M3G) is hydrolysed to free MOR by faecal bacteria in wastewater (86,87); b) Average excretion rate in normal conditions, however large variations have been observed in individuals subjected to MET substitution therapy (34). 6-MAM = 6-monoacetylmorphine, EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.

<i>Administered compound</i>	<i>Major metabolites of interest</i>	<i>Urinary Excretion Rate [%]</i>	<i>Administration Route</i>	<i>References</i>
Morphine	M3G ^a	75	Subcutaneous, intramuscular and intravenous injection	(33,34,86,87)
	Morphine	10		
Heroin	M3G ^a	38	Intravenous injection	(33,34,88)
	Morphine	4		
	6-MAM	1-3		
	Heroin	0.1		
Methadone	Methadone	5-50 ^b (5)	Oral	(33,34)
	EDDP	3-25 ^b (5)		

2.2.4 Cannabis

Cannabis is worldwide the most widespread illicit drug, which is mainly consumed as herb or resin, but also oil (1,2). The major active compound in cannabis is delta-9-tetrahydrocannabinol (THC) and over 20 metabolites have been identified in human urine and faeces (89). Excretion through the latter route has been shown to be the major pathway through which THC is eliminated from the human body (42). The major metabolites encountered after administration of cannabis and their respective excretion rates are reported in Table 4. In WBE studies, THC-COOH has generally been the target compound to monitor consumption of cannabis.

Table 4: Summary of the metabolites and their corresponding excretion rates measured in urine after administration of cannabis. a) Readily oxidizes to form THC-COOH which is more stable (33). THC-COOH = 11-nor-9-carboxy-delta-9-tetrahydrocannabinol; OH-THC = 11-Hydroxy- Δ^9 -tetrahydrocannabinol

<i>Administered compound</i>	<i>Major metabolites of interest</i>	<i>Urinary Excretion Rate [%]</i>	<i>Administration Route</i>	<i>References</i>
THC	THC-COOH	0.5-0.6	Smoke	(33,42,52)
	OH-THC ^a	2		

2.2.5 New psychoactive substances

New psychoactive substances are a group of compounds which have entered the market of illicit drugs in the past two decades and are a growing problem in some European countries (72,90). Some WBE studies have focused on the potential analysis of these compounds in wastewater (91–93), yet there is a lack of pharmacokinetic data making it difficult to determine which compounds to target. In particular, there is little or no literature about the metabolization pathways. However, some recent studies have been published which focus on the identification of metabolites of the more established NPS, like for example mephedrone (94). However, because new substances are constantly being produced and released on the market, it is very difficult if not impossible to keep the pace and conduct thorough pharmacokinetic studies for each of them.

2.3 Wastewater sampling

Sampling represents the initial step of every analytical procedure, however obtaining an exact representation of the object under analysis is almost impossible. Thus, it is extremely important to cautiously design the sampling approach to minimise errors introduced during the sampling procedure. Sewers are a highly variable environment, subject to important variations in flows and pollutant loads (95). Correct sampling procedures are fundamental, especially if one seeks to quantify trace level compounds. In fact, errors caused by incorrect sampling procedures can be orders of magnitude greater than analytical errors (95,96). The uncertainties linked to sampling procedures have been extensively studied since the early works of Gy, who developed the so-called “Theory of Sampling” (TOS) for particulate and heterogeneous materials (97,98). It has been shown that all sampling (intended as a mass reduction procedure) will always introduce errors. Guidelines have been established in order to minimize, or at least control, and quantify these errors. In the context of WBE, where scientists are faced to an extremely complex environment, great care should be taken to select appropriate sampling procedures. Sampling plays an even more important role in comparative studies, where results from different locations are compared to determine if differences in drug use can be identified. Because, as will be discussed in the next section, distinct sampling modes exist and WWTP will implement one of these, according to their logistic and instrumental constraints. Thus, comparing results from different locations should be done with caution.

2.3.1 Sampling practices in WBE

In the field of environmental water analysis, different types of sampling procedures exist, but these can be grouped into two major classes: active (also called “bottle” or spot sampling) and passive sampling. The former consists in direct collection of water samples, processing (i.e., filtration, extraction and concentration) and chemical analysis. The latter consists in exposing a sorbent to the environment (e.g., wastewater stream) for a specific period of time (days up to weeks, depending on the question one seeks to answer). These are then processed to extract the collected compounds.

Passive (integrative) sampling has become an interesting alternative in environmental water analyses since it does not require power sources and the samplers can be deployed for long periods without maintenance. These systems are mainly used to obtain time weighted average (TWA) analyte concentrations. However, these techniques have the disadvantage of needing a calibration step which has to be carried out under controlled conditions, as analyte uptake is influenced among others by pH, temperature, matrix composition and flow rates. Additionally, when deployed to sample raw wastewater, significant clogging and biofouling can occur thus disrupting analyte adsorption. Yet, some convincing results have been reported for applications in WBE (99). In particular, this type of approach could be used to monitor the content of wastewater in sites where deployment of automatic samplers, used in the active approach, is difficult (e.g., domestic sewers and inaccessible manholes). However, to date, applications of integrative samplers in wastewater remains limited and focus will be set on active sampling.

2.3.2 Active Sampling

Active sampling consists in the active collection of wastewater samples directly from the stream. Various ways exist to collect samples and the main distinction that can be made is between grab and composite samples. The former consists in collecting a single point sample at a given moment in time. Although providing a quick snapshot of the composition of wastewater, this approach is not suitable when one seeks to obtain a representative picture of the composition of a complex system like wastewater.

Composite sampling consists of collecting a certain number of subsamples (designated increments), which are then aggregated to form a composite sample. Increments can be collected proportionally to time (i.e., both frequency of collection and collected volume are fixed), to volume (i.e., higher frequency at high flows, and vice versa, but constant sample volume) or to flow (i.e., constant frequency but sampled volume is proportional to flow) (100). On the one hand, composite sampling is more complex and requires the use of an autosampler. On the other hand, if designed correctly, it allows collecting representative wastewater samples, a particularly important feature when the goal is to monitor drug use through wastewater analysis.

2.3.2.1 Sampling frequency

Frequency, referred here as the time between collection of each increment, is a crucial parameter which has to be defined when designing a campaign based on composite sampling. Wastewater streams are constituted of an intermittent flow of discharge pulses and not, as one might think, of a continuous system (100). Thus, concentrations of analytes in wastewater streams are subject to random and systematic variations and ideally one should monitor these fluctuations continuously using an online measurement device (101). Yet, this type of instruments only allow to measure a limited number of compounds or water quality indicators which are present in high concentrations.

Fluctuations in analyte concentrations are due to numerous factors, among which meteorological events (e.g., rain, snowmelt) and, particularly important in the context of WBE, household and sanitary ware.

These are characterised by intermittent flushes, making drug residues likely subject to short-term variations (95). Flushing events can last for a few seconds up to minutes and, because of dispersion in the sewer system, their appearance at the sampling point can last for longer periods. The duration of these pulses, and in particular that of toilets, should be estimated by considering the structure of the investigated sewer. Its architecture (i.e., gravity-drained, pressurized or combined) also influences the dispersion of the pulses. In gravity-drained sewers, pipelines are placed so that flow occurs only through the effect of gravity and water is not stocked during transportation to the WWTP. The situation is different with pressurized sewers because of the activity of pumps. In these systems, wastewater is collected in wells until a certain volume is reached and when this occurs, wastewater is pumped into pressurised pipes. This cycle is repeated once the critical volume in the well is reached again. Clearly, these cycles influence the composition of wastewater reaching the WWTP and should be taken into account when planning a sampling campaign (95). In particular, the sampling frequency should be selected so to be adapted to pumping cycles (ideally shorter than such cycles).

Regardless from the architecture of sewer system under investigation, the sampling frequency should be selected in order to account for the fluctuations in analyte concentration. The lower the occurrence of the target analyte, the higher the sampling frequency to minimize sampling errors. In a study by Ort and Gujer (102), the authors conducted simulations to evaluate the deviation of benzotriazole loads (expected to be present in 50 pulses per hour) in 2h-composite samples using two different sampling frequencies (i.e., 5 and 15 minutes). Using the first sampling frequency, the estimated loads deviated $\pm 10\%$ from the real value, while with the second, the deviation increased to $\pm 25\%$. These results show that different sampling frequencies can have substantial impact on the estimated loads. However, the authors found that for a given compound (present in a given number of flushes per hour), increasing sampling period (by sampling for 24h instead of 2h), smaller deviations were observed at equal sampling frequency. Nonetheless, in larger sewer systems (e.g., city), each relevant toilet pulse will, from the moment it enters the system until it reaches the sampling point, have been mixed with large volumes of wastewater. Apart from diluting, this will also disperse the analyte in the

sewers, thus reducing sampling errors as the risk of missing the relevant pulse decreases (102).

The elements discussed in this section show that the sampling frequency plays a crucial role in the representativeness of the obtained data and that it should be selected carefully. When no information is available concerning the number of relevant flushes, a preliminary study to monitor short-term variations (using high frequency sampling) should be carried out in order to determine an appropriate sampling frequency (100,103). If information is partly available (e.g., prescription data or self-reported surveys), the number of relevant pulses can be estimated and an appropriate sampling frequency can be selected using mathematical models. However, it should be noticed that each sewer system presents individual characteristics and, if possible, a preliminary high-frequency sampling campaign should be set up (95). Yet, expensive and time consuming instrumental analysis often dissuade from conducting these.

2.3.2.2 Sampling modes

Among the different types of sampling modes available, i.e., time-, volume- and flow-proportional, the latter should be favoured as it is the only one which allows to correctly weigh the collected increments, in particular when trying to estimate average trace level concentrations (95). Because in volume-proportional sampling, it is the frequency that changes and not the volume, collected increments are not properly weighted, thus hindering the measurement of the true average concentration. This is clearly also the case for time-proportional sampling, where both the frequency and the volume are held constant (95). Regardless of the type of sampling approach used, sampling errors increase as the sampling frequency and the number of relevant wastewater pulses decrease. Flow-proportional sampling can be performed in two modes: continuous and discrete. In the first case, part of the stream is redirected to generate a proportional side-stream (104). Although being the ideal approach, it is rarely applicable because it requires often complex interventions to deviate part of the stream. Thus, discrete sampling is often preferred because more simple and cost effective. The selection of the sampling frequency depends on the question which one seeks to answer. If the aim is to evaluate short-term fluctuations, then the temporal resolution needs to be high (i.e., minutes). Otherwise, if one aims to monitor changes during longer periods (e.g., weeks), and if short-time or daily variations are negligible, lower resolutions could be enough (95). Yet, conservative approaches using high sampling frequency should be considered if possible.

2.3.2.3 Flow measurements

Flow measurement is another crucial parameter when collecting wastewater samples to assess analyte mass loads. Two types of flows are described in the literature: open-channel and close conduit flows (105). The main difference between the two is that the former one has an open surface to the atmosphere (e.g., open channels or pipelines not completely filled). In this kind of systems, the only force that can generate flow is gravity, while if the pipe runs full, the pressure in the conduit becomes different from the atmospheric pressure (105). A very detailed literature exists about the different types of instruments used to measure open-

channel and pressurized flows (105–107) and detailing them all goes beyond the scope of this work. However, it is important to highlight that correctly measuring wastewater flows is a complex task and errors of up to 20% have been reported (108,109). When assessing substance loads, uncertainties linked to flow measurements should imperatively be included in the calculations.

2.3.2.4 Sampling locations

The sampling location depends of the type of information that one seeks to collect and an important distinction was previously made between open (e.g., large communities) and confined (e.g., specific cohorts) environments. This also influences the sampling strategies which have to be implemented. In the case of house connections (close environments), flows are dramatically reduced, intermittent or even inexistent. This can be a challenging situation when using automated samplers, which often require a minimum water height to operate properly. Furthermore, flow rate measurements become very difficult in these conditions, thus introducing large uncertainties in the estimated mass loads. However, if the sampling point is easily accessible, it could be possible to deviate the main stream to obtain a side-stream and conduct continuous flow-proportional sampling (104). In most WBE applications, sampling was carried out at the inlet of a WWTP, mainly because the objective was to monitor the consumption of a large population. In particular, samples should be collected prior to any treatment process to minimize potential losses/transformations. These locations have the advantage of being easily accessible and, often, automatic samplers are readily available.

2.4 Analysis of wastewater samples

The analysis of drug residues in wastewater samples involves numerous steps which go from collection on site, to processing in the laboratory and finally analysis using various instrumental techniques. A summary of the various steps, which will be discussed in this section, is reported in Figure 2.

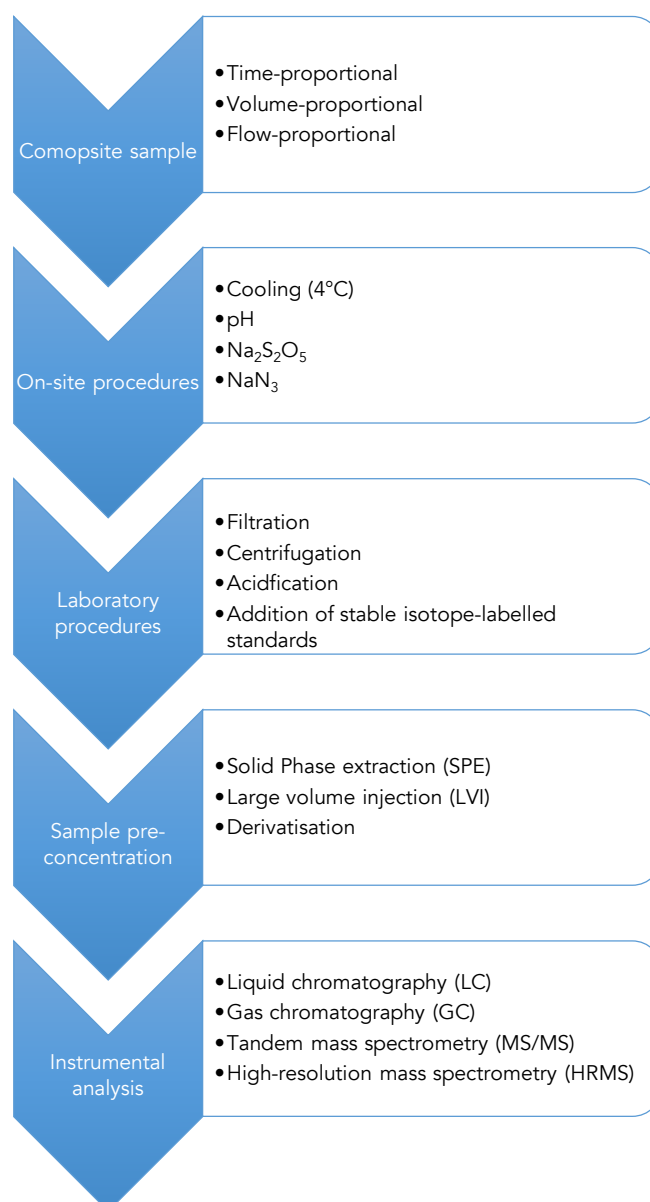


Figure 2: Scheme of all steps involved in the collection, processing and analysis of wastewater samples for the quantification of drug residues.

2.4.1 Sample pre-processing

Wastewater-based epidemiology relies on methods which allow to extract, detect and quantify analytes in the low nanogram per litre range in an extremely complex matrix. Thus, extreme caution has to be taken to minimize the risk of further modifying the target compounds on site and once the samples have been transported to the laboratory for chemical analysis.

Numerous studies have been carried out to assess the potential modifications which can occur to the target analytes in sewers but also in the laboratory.

2.4.1.1 On-site procedures

In WBE applications, wastewater samples are mostly collected over a period of 24 hours, since the objective is to obtain a sample which is representative of a whole day. Because samples are collected only once the sampling process is completed, part of the sample will have spent almost 24 hours in the reservoir before being transported to the laboratory. To reduce potential degradation, different expedients have been suggested in the literature. The first and most widespread mean consists in cooling the samples on site, which is easily achievable as most autosampler allow cooling down to 4°C. Other approaches suggested are acidification (110,111), addition of sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) (112) or sodium azide (NaN_3) (58). These are particularly interesting because they can be added to empty bottles prior to sampling and thus can minimize degradation already on site. Sodium metabisulphite and sodium azide are especially adapted for this as they are in powder form and less dangerous compared to strong acids. Moreover, the former additive has been shown to significantly reduce the degradation of almost all drug residues, included more fragile compounds such as 6-MAM (112).

2.4.1.2 Laboratory procedures

After being transported to the laboratory, samples have to be further processed prior to extraction and analysis. Generally, this consist in removing particulate matter, which would otherwise cause clogging during pre-concentration and analysis. Focus has always been set on wastewater itself because it was considered that the target analytes are fully dissolved. However, various studies have been carried out which showed that for some drug residues, an important proportion tends to be adsorbed on to suspended soils (113–117). Highest adsorption rates were reported for methadone and its metabolite EDDP (i.e., 7-15% and 8-30% of the total measured loads, respectively), while other substances were generally in the range 2-8% of the total loads. However, analytes might also adsorb onto plastic containers used to collect wastewater samples, which has been called into question as an explanation for losses of THC-COOH (118,119). The results from these studies are highly relevant because they allow to estimate, in those cases where suspended soils were not analysed for illicit drugs, the proportion of drug residues which are expected to be adsorbed. Removal of particulate matter is usually carried out by filtration of samples, either by percolation under gravity or under pressure, through a series of glass and/or nitrocellulose filters or via centrifugation (120–122). For those analytes which are fully dissolved in the liquid part of the sample, this procedure has been shown to have a positive effect on their stability (121). Subsequently, the pH of the samples is further adjusted to the necessary values depending on whether the samples are further pre-concentrated or analysed directly. However, before any additional step is carried out, stable isotope-labelled internal standards are added to the samples. These are used to account for losses during extraction and are used as internal standards for quantification. Nonetheless, adding internal standards before filtration and/or

acidification should be contemplated for those analytes whose concentration could be altered by these procedures (e.g., sorption, hydrolysis).

2.4.2 Stability of target analytes

Illicit drug residues found in wastewater are exposed to a very complex environment which has been shown to have an impact, more or less pronounced, on these compounds. The major phenomenon which can take place both in sewers but also once the samples have been collected is that the analytes undergo further degradation. If not minimised, this can substantially bias the results and thus hinder the correct interpretation of the obtained results. The determining factors which have been identified as increasing the degradation are temperature, pH and microbial activity.

Concerning temperature, studies were conducted to assess the stability of target analytes at ambient temperature, which is supposedly representative of the environment to which they are exposed during transportation in sewers, as well as at 2 or 4°C which are the storage temperatures generally found in automatic samplers deployed on site. Results showed that substances most affected by higher temperatures are heroin, its metabolite 6-MAM, which are rapidly transformed to morphine, and cocaine, which is transformed to benzoylecgonine and its other its metabolites (59,86,123). These further transformation will result in overestimations of the actual concentration of metabolites. Thus, it is very important to reduce these degradations by, for example, using refrigerated autosamplers and adding stabilisers to empty bottles prior to sampling (see below). Yet, if residence times of wastewater in sewers are long, in-sewer degradation is likely to occur and little can be done to prevent this. This seems to be less the case for amphetamine and its derivatives, which have been shown to be relatively stable, even at ambient temperature (58,59,119,123).

As already discussed above, pH has a strong influence on the stability of drug residues. Acidic conditions (pH ~ 2) have been shown to be promote the stability of drug residues (121). Yet, pH values encountered in sewers are generally around 7.5, which negatively influences the stability of the target compounds (121). Particularly affected substances are opiates (heroin and 6-MAM), as well as cocaine, while ATS are again quite stable (58,121). Exception to this is THC-COOH which as been shown to be stable in sewer conditions but is rapidly lost, likely due to adsorption, at pH = 2 (118,124).

The final factor which influences the stability of drug residues is microbial activity, which tend to further metabolise drug residues. In sewers, analytes are exposed to biofilms, extremely rich in microorganisms, which will further metabolise these substances. While this effect as been for long highlighted, its impact is still undetermined. Studies are currently being carried out to determine to which extent exposure to biofilm influences the stability of drug residues and its impact on the measured concentrations (119). The amplitude of the microbial activity can be attenuated by the addition of sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) and sodium azide (NaN_3) once samples have been collected (58,112).

Long term storage of wastewater samples has also been investigated as samples cannot always be analysed immediately after collection. Although storage at -20°C of acidified samples has been shown to prevent degradation for up to three weeks (125), this is not very

practical if a larger number of samples has to be stored. An alternative and less cumbersome option is to extract the samples on solid-phase extraction (SPE) cartridges and to store these at -20°C; studies have shown no losses in analytes for up to 6-12 weeks or more (58,121,126).

2.4.3 Sample pre-concentration

2.4.3.1 Solid-phase extraction

Solid-Phase Extraction (SPE) is the most widely used clean-up and sample pre-concentration technique for the analysis of drug residues in wastewater. It has been used with both liquid chromatography (LC) and gas chromatography (GC) (25,58,63,86,120,127). SPE is a separation technique based on adsorption of analytes, contained in a mobile phase (the liquid sample), onto a stationary phase (the sorbent). Commercially available phases are separated in 3 major classes, i.e. reversed, normal and ion exchange phases (128,129). The nature of the target analytes and the samples matrix guide the selection of the appropriate stationary phase. In most cases, this is characterized by a silica support whose surface is then modified by reaction with specific compounds (e.g., alkyl C-18 chains) to obtain specific properties (129). In the field of WBE, numerous types of sorbents have been tested and detailing them all goes beyond the scope of this work. However, it should be noticed that the best overall recoveries were generally obtained with ion exchange cartridges (e.g., mixed-mode cation-exchange, Oasis® MCX, Waters, Milford, MA, USA) in acidic conditions. More universal reverse-phase sorbents (e.g., Oasis® HLB Waters, Milford, MA, USA) have also been implemented because they allow to extract a broader spectrum of substances. As will be discussed further on, this is particularly interesting when using screening approaches based on high-resolution mass spectrometry (HRMS) (130,131).

2.4.3.2 Large-volume injection

Although being very efficient and widely used, SPE is time consuming and requires additional sample manipulations which could introduce further errors. To overcome this issues, some authors have developed so called large-volume injection (LVI) methods (57,125). These methods have gained greater interest through the ever growing sensitivity of mass spectrometers (MS). After filtration, an aliquot of the sample is generally taken and further diluted, to reduce matrix effects, prior to injection. However, dilution and matrix effects still prevent the efficient use of this approach for the analysis of all types of compounds and SPE still remains the most widespread approach in WBE.

2.4.4 Instrumental analysis

Detection and quantification of trace level compounds in complex aqueous matrices such as wastewater, require the use of sophisticated and sensitive analytical instrumentations. Amongst the available techniques, scientists have generally implemented LC and GC methods, coupled to various types of mass analysers.

Liquid chromatography is by far the most applied method in WBE, mainly because target analytes are often polar compounds largely dissolved in the liquid phase. LC applications for

the analysis of illicit drugs in wastewaters were mainly based on the use of reverse-phase columns combined to a mobile phase composed of a mixture of water and an organic solvent, mainly methanol and acetonitrile (122). Hydrophilic interaction liquid chromatography (HILIC) has also been implemented (64,111). Gas chromatography has also been successfully implemented for the analysis of drug residues in wastewater (58,76,132,133), however its application is less widespread because it requires derivatisation of the target analytes.

The different chromatographic methods have always been coupled to mass spectrometric detectors. In particular, tandem mass spectrometers (MS/MS) have been widely implemented because of their high sensitivity and selectivity (29,65,111). However, the major drawback of this type of mass analyser is that, to take full benefit of its sensitivity, it requires prior knowledge of the target compounds, thus precluding wide spectrum screening approaches. However, an increasing number of studies have been carried out in recent years using HRMS instruments as these have now reached sensitivities similar to MS/MS instruments (134). The major advantage of these instruments is that they could potentially be used for non- or semi-targeted screening approaches, thus enabling to determine the presence of an analyte without having to pre-select the target compounds (131). Furthermore, retrospective analyses can be carried, without the need to re-inject the sample. Yet, some limitations still exist regarding the sensitivity of these instruments in complex matrices such as wastewater, but technical improvements and further applications will make HRMS one of the method of choice for the analysis of illicit drugs and other compounds in environmental matrices (134).

2.5 From measured concentrations to indicators of drug use

In the previous sections, the different physiological and environmental parameters which make that illicit drugs and their metabolites can be detected and measured in wastewater samples have been exposed. Additionally, sampling modes, sample preparation and instrumental techniques used to analyse wastewater samples and determine concentrations of drug residues have been presented. Although giving an indication of the presence, and thus the consumption, of a specific illicit drug, concentrations are still not a useful indicator of the extent and patterns of drug use. In the following sections, the various indicators of drug use which can be derived from wastewater analysis will be presented.

2.5.1 Absolute and population normalised loads

From concentrations measured in wastewater samples, scientists have generally estimated absolute mass loads. These can be obtained by simply multiplying the concentrations of drug residues measured in composite samples (generally in the nanogram per litre range) by the flow (in litres or cubic meters). The obtained value can be used to monitor the occurrence of drug residues over time increments and is particularly useful when the focus of a study is on one specific catchment. However, when the focus is to compare results obtained from different locations, interpretation of absolute figures is more difficult as these do not account for the different size of the investigated population. For this reason, scientists have often appealed to so called population normalised loads or per capita loads (135,136). These are obtained by

dividing absolute mass loads by the size of the sampled population and are generally expressed in terms of milligrams per day per thousand inhabitants. Figures about the number of inhabitants are generally provided by census, by the number of registered inhabitants or by the design capacity of the WWTP (122). Yet, these are estimates of the *de jure*, not of the *de facto* population. Moreover, they are static descriptors while the actual population is dynamic: fluctuations occur during time, at several levels, due to e.g. commuters or seasonal activities (137). Besides, estimates derived from design capabilities of WWTP are likely biased estimates of the *de jure* population (138). In WBE, the *de facto* population is of interest, because this includes all people, resident or not, which are contributing to the analysed wastewater samples (137). Various studies have thus been carried out to develop anthropogenic markers which could be used to estimate the size of the *de facto* population and monitor its fluctuations.

2.5.1.1 Anthropogenic markers

Water quality indicators have been among the first markers used to estimate the size of the population (53). These are parameters like phosphorous (P), nitrogen (N), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) and are commonly measured by WWTP personnel to assess pollutant loads and the efficiency of the treatments. Studies have been carried out to assess population equivalents (PE) (i.e., amounts produced per day per inhabitant or per thousand inhabitants) which are then used to design WWTP capacities (139–141). While easily accessible because they are routinely measured at WWTP, these indicators have a major drawback, they can be influenced by non-human sources such as industrial or agricultural discharges (138). Another, less affected water quality indicator which has been used in this research is ammonium ($\text{NH}_4\text{-N}$) (76), as will be discussed in *Chapter III*.

Other indicators which have been suggested in the literature are markers which are directly related to human metabolism. Chiaia et al. (125) used creatinine as a biomarker, which was analysed by LC-MS/MS in the collected samples. However, uncertainties related to the use of creatinine arise from the existence of non-human sources (e.g., foods) (137) and the instability of the molecule in wastewater (142). In a further study by Chen et al. (142), additional compounds (i.e., creatinine, cholesterol, coprostanol, cotinine, cortisol, androstenedione and 5-hydroxyindoleacetic acid (5-HIAA)) were investigated as potential population biomarkers. 5-HIAA, a neurotransmitter, was deemed the most suitable population biomarker. Unfortunately, no further applications where the latter was used have been reported since. In an extensive study, the potential use of pharmaceuticals and personal care products as population biomarkers was investigated (138,143,144). Using a combination of 14 chemicals and a sophisticated Bayesian model, the authors were capable of obtaining highly accurate estimates of the size of the sampled population. However, wastewater samples were collected simultaneously in different locations during census day, thus meeting all this criteria in another location or country is difficult. Yet, the major advantage of the proposed method is that it uses multiple markers simultaneously, providing a more accurate estimate.

Regardless of the nature of the marker, there is one common advantage in using these to compute normalised drug loads, namely that they will allow to overcome uncertainties due to

systematic errors in flow measurements, as well as to account for eventual losses in sewers (135,138).

2.5.2 Back-calculations

The final step in WBE consists in estimating the amounts of parent compound initially consumed based on measured mass loads of drug residues. This is done by taking into account the excretion rate of the target analyte and the mass ratio between the parent compound and the metabolite, if mass loads of the latter are being used for the calculations. This approach, which is shown in Eq. 1, was first implemented by Zuccato et al. (28) to estimate cocaine consumption from measured benzoylecgonine loads.

$$Consumption \left[\frac{g}{d} \right] = \left(Conc \left[\frac{ng}{L} \right] * Flow \left[\frac{L}{d} \right] * \frac{1}{Excretion[\%]} * \frac{Mwc}{Mwm} \right) * 1e^{-9} \quad \text{Eq. 1}$$

where *Consumption* represents the estimated amount of pure substance consumed per day; *Conc* is the analyte concentration measured in the collected samples (the parent compound itself or a metabolite); *Flow* is the daily flow; *Excretion* is the proportion of the initial dose which is found in urine or faeces (or both) as target analyte (generally after 24 hours) and *Mwc* and *Mwm* are the molecular weights of the parent compound and the metabolite, respectively. The latter factor can be omitted when the parent compound is used as marker. By further considering the average dose of a consumption unit (e.g., 100 milligrams of pure cocaine per dose (145)), the extrapolation can be expanded to estimate the number of doses used.

2.5.3 Uncertainty assessment

Performing back-calculations has been subject to numerous discussions among researchers. One of the major concerns which has been mainly raised by toxicologists is that large intra- and inter-individual variations in excretion rates have been observed (122), as discussed in *Section 2.2*. Furthermore, the issue of the limited number of patients included in the trials or the unrealistic administration routes, have also been raised as potential flaws in the validity of these studies (122). Yet, a wide array of techniques have been developed to combine results from different studies to obtain a pooled, eventually more accurate, estimates of the parameter of interest. Moreover, for those substances where a relatively large number of studies have been carried out, because the number of individuals included in WBE campaigns is large (i.e., hundreds or thousands potential drug users), it is plausible to assume that excretion rates will tend to average values. Still, if the estimated excretion rate has a large uncertainty because the number of studies is limited, it is dose depended or various administration routes can be contemplated (e.g., cocaine, heroin and methamphetamine), all which having different excretion rates, this will strongly influence the final estimate.

As discussed in the previous sections, there are additional sources of uncertainty, some of which are more difficult to control or estimate, that will affect not only back-calculations but

also absolute and population normalised loads. These were extensively discussed in a review by Castiglioni et al. (146), a summary of which is shown in Table 5.

Table 5: Sources and estimated uncertainties identified by Castiglioni et al. (146).

<i>Step</i>	<i>Causes</i>	<i>Uncertainty [%]</i>
Sampling	<ul style="list-style-type: none"> • Sampling mode • Frequency • Catchment characteristics 	5-10
Analytics	<ul style="list-style-type: none"> • Sample preparation • Method performances 	1-34
Stability of drug residues	<ul style="list-style-type: none"> • Residence time • On site storage and pre-processing • In laboratory storage 	< 10
Estimates of population size	<ul style="list-style-type: none"> • Use of inadequate data sources 	7-55
Back-calculations	<ul style="list-style-type: none"> • Limited pharmacokinetic data 	Substance dependent

The impact of these sources of uncertainty is the highest in comparative studies, because different sewer architectures, sampling approaches and analyte stability will play an important role. These are further exacerbated when the number of collected samples is limited. Contrarily, some factors can be regarded as constants when the focus of the campaign is to monitor one location over longer periods. The reason being that sampling will likely be performed using the same approach across the whole sampling period, sewers architecture will not change (or not significantly) and, consequently, the factors which influence the stability of analytes will remain constant. These aspects, which will be discussed further in *Chapter IX*, are the main reasons why WBE will provide the most reliable results when applied to monitor single locations over longer periods of time.

Following the identification of these sources of uncertainty, numerous critical discussions have taken place among researchers about the validity of the obtained results. Initially, it was thought that these would preclude the use of wastewater data for comparative studies, due for example to differences in catchment characteristics, residence time and sampling approaches. Similarly, back-calculations could not be performed because too many factors account for uncertainty. However, the existence of these uncertainties does not hinder the use of wastewater data as long as they are taken into account in the calculations and clearly stated in the interpretation of the results. This has recently pushed the development of formal statistical frameworks, based for instance on Monte Carlo simulations or Bayesian inference models implemented using Markov Chain Monte Carlo methods (143,147). Such approaches allow taking into account the interaction of the different sources of uncertainty, include prior knowledge about the parameters and compute realistic estimates of the parameters and their uncertainty.

III Population normalization with ammonium in wastewater-based epidemiology: Application to illicit drug monitoring

This chapter is based on the following publication:

Been F., Rossi L., Ort C., Rudaz S., Delémont O., Esseiva P., *Population Normalization with Ammonium in Wastewater-Based Epidemiology: Application to Illicit Drug Monitoring* Environmental Science & Technology, 2014, 48 (14), 8162–69; <http://dx.doi.org/10.1021/es5008388>

For the complete publication see *Annexe I*.

The importance of developing means to account for fluctuations in the size of the sampled population when monitoring illicit drug use via wastewater analysis has been highlighted in several studies. In particular for long-term and comparative campaigns, it is important to know whether the observed patterns are due to changes in consumption habits (e.g., higher or lower consumption, substance purity, policy changes and prevention campaigns) or if these are caused by a third variable, namely an increase (or a decrease) in the size of the sampled population. As already discussed in *Section 2.5.1.1*, census based data or WWTP capacity are static values and do not allow to account for short-term population dynamics (e.g., commuters) (137). Besides, markers of human metabolism or pharmaceuticals (cf. *Section 2.5.1.1*), often require different or additional sample preparation steps and are thus time-consuming.

Ammonium, an indirect marker of urine content in wastewater (38,40), could be a viable alternative to existing markers. Its occurrence in sewers is largely due to the hydrolysis of urea (148) and it is mainly introduced in via toilets (39). Furthermore, it is expected to be less influenced by non-human sources compared to conventional water quality indicators (53) and it can be measured online.

The objective of this part of the research consisted in investigating if ammonium loads ($\text{NH}_4\text{-N}$), measured directly in the wastewater stream, could be used i) to obtain estimates of the size of the population and capture its fluctuations at different time scales (i.e., weekly and monthly), ii) to determine if a correlation could be established between diurnal patterns of $\text{NH}_4\text{-N}$ and target illicit drug residues, and iii) determine if and how fluctuations in the size of the samples population (measured with $\text{NH}_4\text{-N}$) affect patterns of illicit drug residues measured in wastewater.

3.1 Ammonium as population marker

Data consisted of ammonium concentrations (in mg/L) measured between January and October 2013 at the WWTP of the metropolitan area of Lausanne (220'000 inhabitants in 2013 (149)). Concentrations ($\text{NH}_4\text{-N}_{\text{ISE}}$) were measured using an ion-selective electrode (ISE)

directly immersed in the wastewater stream. An image of the probe is shown in Figure 3. Using flow data (in m³) provided by the WWTP personnel, hourly and daily profiles of NH₄-N loads were computed. These were then examined to determine if changes between week- and weekend-days could be observed and if differences due to weather could be highlighted. Results are shown in Figure 4.



Figure 3: Illustration of the ion-selective electrode used to measure ammonium online (AmmonLyt® Plus 700IQ, WTW GmbH, Weilheim, Germany). 1) Protective hood, 2) Temperature probe, 3) Support and electrodes, 4) Sensor shaft and 5) Plug head connection. Reproduced with the kind permission of WTW GmbH, Weilheim, Germany (AmmonLyt® Plus 700IQ, user manual, fig. 1-2, page 1-2).

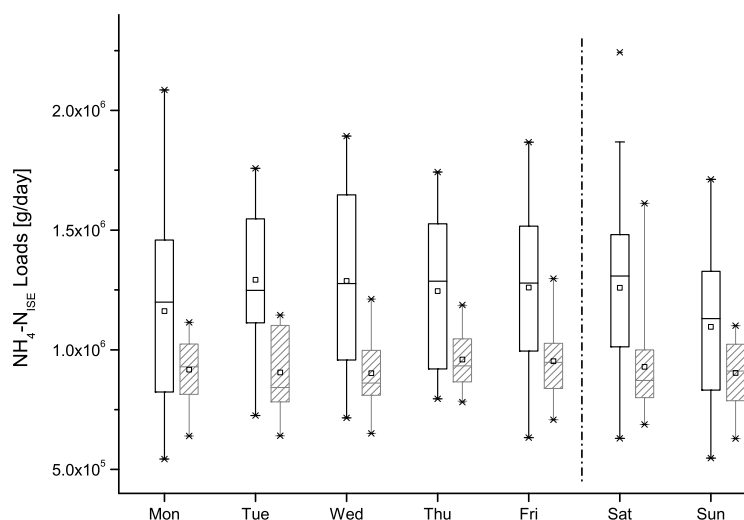


Figure 4: Boxplot of daily NH₄-N loads (g/day) measured with the ISE (NH₄-N_{ISE}) during dry (black) and rainy (grey) weather. Taken from Been et al. (76).

As can be seen, during dry weather, loads measured on Sundays were on average 6.5– 16% lower compared to other days, which was subsequently confirmed by statistical analysis. Using available official statistics about commuters (150), the net increase in the size of the population expected during a week-day was calculated. The latter was estimated to at least 15% of the total population (i.e., 34'000 individuals), which is in agreement with results from NH₄-N. Yet, decreases in NH₄-N were observed only on Sundays and not Saturdays. One potential explanation for this is that on Saturday the proportion of non-residents is still significant (e.g., people coming to the city centre for leisure).

Using total daily NH₄-N loads, estimates of the size of the contributing population were computed. This was achieved using a population equivalent of 6.9 ± 0.4 g/day of NH₄-N, i.e. the estimated amount of NH₄-N excreted per person on average each day. This value was derived from a nationwide survey aimed at determining pollutant loads in various WWTP

across Switzerland (see the *Supporting Information* of the article presented in *Annexe I* for further details). Results for the period January-October 2013 are shown Figure 5. Over this period, the average population was estimated to 190'000 inhabitants (minimum, 78'000; maximum, 479'000; standard deviation, 58'000), which is approximately 15% below estimates obtained with other water quality parameters (i.e., COD and P). During summer months, lower estimates were obtained, likely due to summer holidays and the lower number of students. In fact, the University of Lausanne and the Ecole Polytechnique Fédérale de Lausanne (EPFL), which make up for more than 10% of the total contributing population (i.e., 9'000 employees and 22'300 students (151,152)), are also served by the WWTP. Interestingly, an increase in $\text{NH}_4\text{-N}$ loads was observed towards the end of August and the beginning of September, which corresponds to the end of summer holidays. These results illustrate that fluctuations in $\text{NH}_4\text{-N}$ loads, within the same week or during longer periods of time, reflect changes in the size of the contributing population.

With regard to these observations, two approaches, which could be used in WBE studies to monitor consumption patterns, can be suggested: i) use absolute loads of drug residues or ii) compute normalised loads. In the latter case, further two approaches can be suggested: i) divide drug loads by $\text{NH}_4\text{-N}$ loads or ii) use $\text{NH}_4\text{-N}$ loads to estimate the size of the contributing population and compute population normalised drug loads. The first approach could be used if no information about population equivalents of $\text{NH}_4\text{-N}$ or potential non-human sources are available.

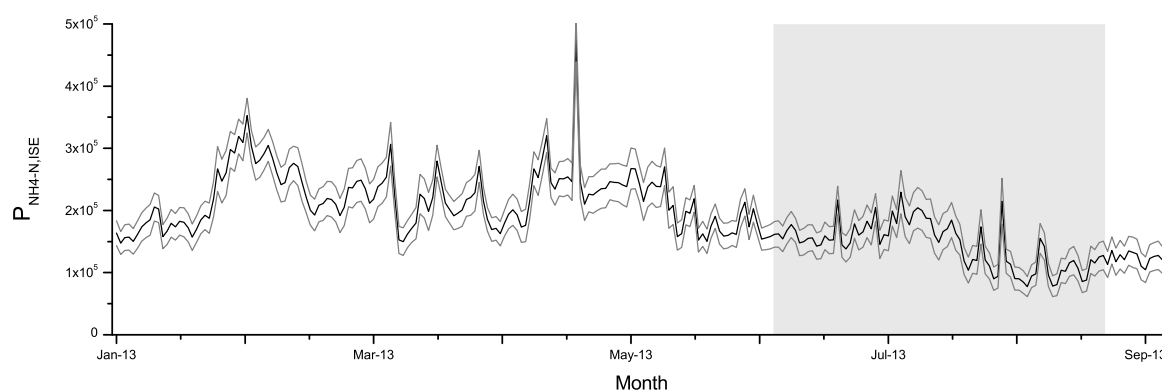


Figure 5: Estimated number of individuals based on $\text{NH}_4\text{-N}$ loads and the associated standard deviation (grey lines). The summer break is indicated by the grey zone between June and September. Taken from Been et al. (76).

3.2 Illicit drug consumption patterns

Initially, diurnal illicit drug patterns were investigated by measuring hourly wastewater samples collected on a week-day (Tuesday to Wednesday) and a weekend-day (Saturday to Sunday). Results are reported in Figure 6-Figure 8. Higher cocaine and benzoylecgonine loads were measured during the weekend, as shown in Figure 7a. During the night from Tuesday to Wednesday, higher cocaine concentrations compared to benzoylecgonine were measured, whilst pharmacokinetic data indicates that the contrary should be measured (33). A similar observation was made on Sunday morning, between 1a.m. and 2 a.m. Although unlikely,

direct disposal of cocaine in sewers could be a possible explanation for these observations. Correlation analysis showed that benzoylecgonine and $\text{NH}_4\text{-N}$ were highly cross-correlated (0.8 to 0.9 for weekend end week-day, respectively), while this was not the case for cocaine (0.2 for both days), suggesting that the metabolite is more closely linked to urine content compared to the parent compound. During the weekend, a shift in the appearance of the morning peak of $\text{NH}_4\text{-N}$ (see Figure 6), cocaine and benzoylecgonine was observed, suggesting that people tend to wake up later. Furthermore, because people likely remain active longer during the weekend nights, cocaine and benzoylecgonine loads decreased later during the night. Normalised benzoylecgonine loads almost constantly increased throughout Saturday evening/night (see Figure 7b). Interestingly, after the 4-5 a.m. peak, a steady decrease was observed. This could be linked to the closing time of nightclubs (e.g. 5 a.m.), located in the mainly gravity drained city centre, and people going home.

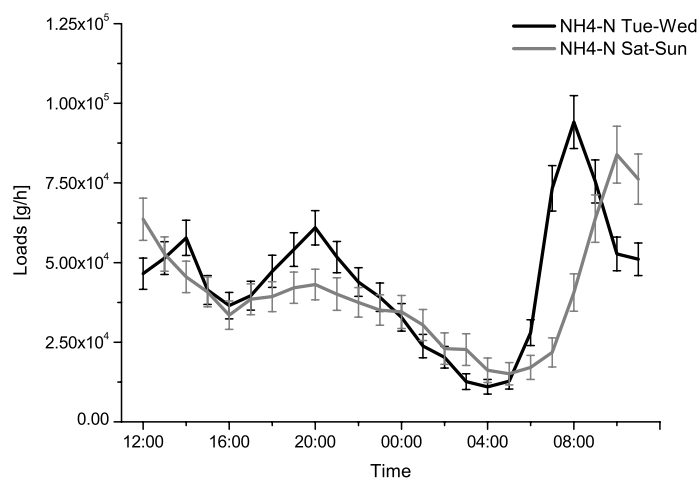


Figure 6: Diurnal profiles of $\text{NH}_4\text{-N}$ measured during the week (Tuesday and Wednesday) and weekend (Saturday and Sunday). Taken from Been et al. (76).

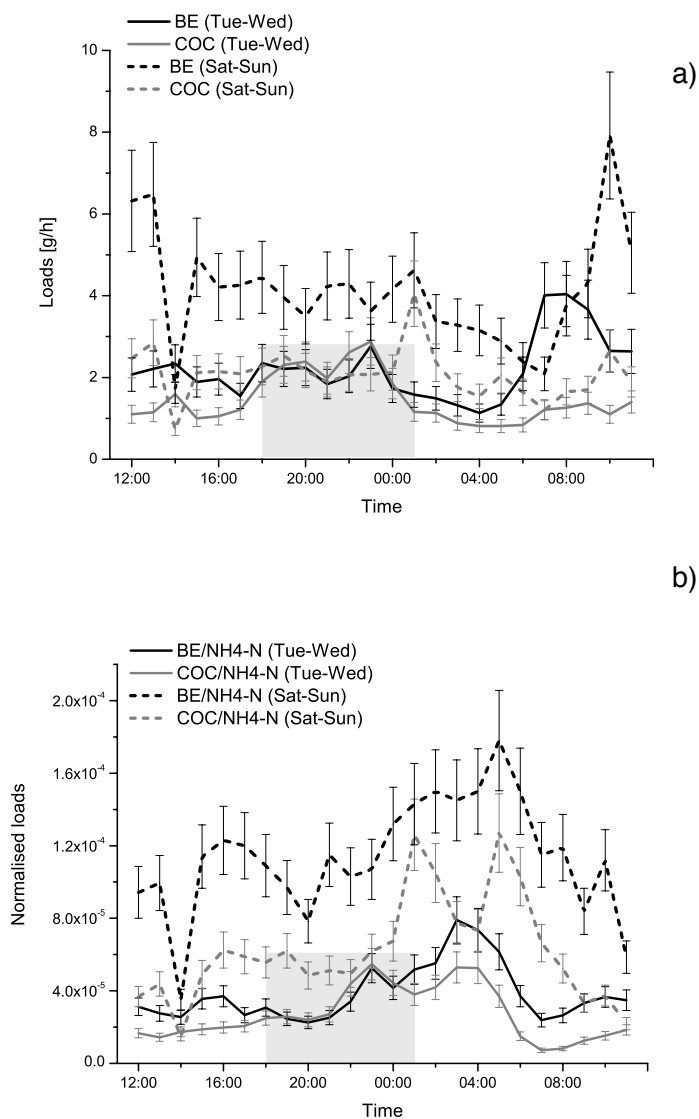


Figure 7: a) Absolute and b) population normalised (based on NH₄-N measurements) cocaine and benzoylecgonine loads measured during the week and the weekend from 12 p.m. to 12 p.m. The grey area indicates the period during which the cocaine/benzoylecgonine ratio was close or greater than 1. Taken from Been et al. (76).

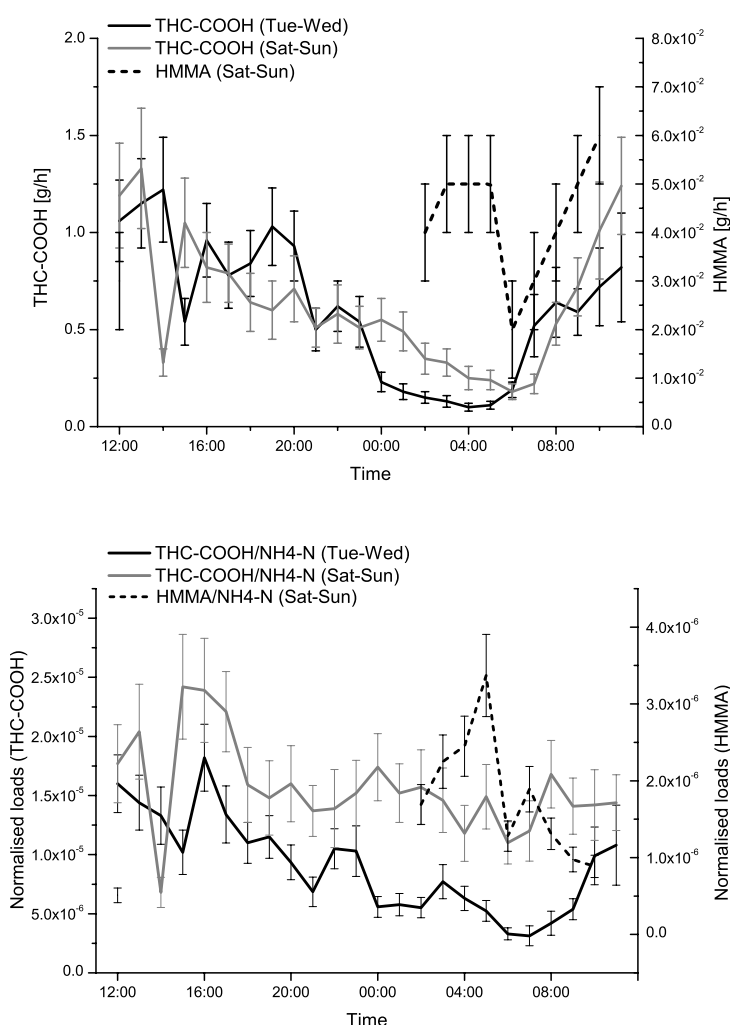


Figure 8: a) Absolute and b) population normalised (based on NH₄-N measurements) THC-COOH and HMMA loads measured during the week and the weekend from 12 p.m. to 12 p.m. Taken from Been et al. (76).

4-hydroxy-3-methoxymethamphetamine (HMMA), one of the major metabolites of MDMA, could be detected only in samples collected on Saturday (see Figure 8). It was detected in the 12 p.m. sample and then only from 2 a.m. of Sunday morning. Loads were stable until 5 a.m. and then, analogously to benzoylecgonine and cocaine, a steep decrease was observed. According to pharmacodynamic studies, measured HMMA concentrations could be due to a recent consumption, as the metabolite is formed rapidly and is detectable in urine as soon as 0.83-3.33 hours after consumption (153). Population normalised loads showed the same pattern as observed previously for benzoylecgonine and cocaine.

For THC-COOH (see see Figure 8a), loads steadily increased during the morning and the beginning of the afternoon. This distinct pattern could be linked to the particular metabolism of THC-COOH, which reaches maximum urinary concentrations between 6 and 10h after smoking (154,155), compared to benzoylecgonine and cocaine which reach peak concentrations sooner (5-8h and 4-6h, respectively(156)). The non-resident population, whose contribution to measured THC-COOH loads would be observable only later, could be

another explanation to these patterns. Normalised loads (see see Figure 8b) were higher between Saturday and Sunday compared to Tuesday to Wednesday. Still, on both days, highest values were measured in the late afternoon. These then decreased during the evening and some sporadic peaks were observed between 10 p.m. and 5 a.m. Similarly to what was already observed for benzoylecgonine and cocaine, THC-COOH loads decreased less rapidly during the weekend night. Again, this could be due to people being active longer.

Census and $\text{NH}_4\text{-N}$ -based normalised loads were computed to determine if the latter provided additional information which could help interpreting the observed patterns. Results are reported in Figure 9. In agreement with findings from previous studies (46,157,158), benzoylecgonine and cocaine loads were always higher during the weekend compared to week-days.

Normalising data by population estimates derived from $\text{NH}_4\text{-N}$ resulted in an increase of calculated loads compared to census based estimates, in particular for samples collected during the weekend. However, except for the 15th of June, no particular difference in the overall pattern could be observed compared to absolute or census-normalised loads. The difference in loads measured between Tuesday/Wednesday and Saturday/Sunday samples within the same week was greater when $\text{NH}_4\text{-N}$ estimates were used to normalise loads. Yet, these difference were not statistically significant (p-value of 0.1 and 0.17 > $\alpha = 0.05$ for benzoylecgonine and cocaine, respectively). Similarly to cocaine and benzoylecgonine, normalising HMMA loads with $\text{NH}_4\text{-N}$ -based estimates did not modify the overall pattern which, in agreement with recreational use of MDMA (158), was measured only between Saturdays and Sundays. In the case THC-COOH, both absolute and normalised loads measured during the weekend were systematically below those measured during week-days (i.e., between 3 and 27% lower). While being in contradiction to previous findings, suggesting that cannabis is consumed regularly across the week (46), these observations could be due to a smaller non-resident population. Still, no statistically significant difference could be highlighted between week-days and weekends (Mann-Whitney test; p-value of 0.34 > $\alpha = 0.05$). Similarly to results obtained for benzoylecgonine, cocaine and HMMA, normalisation through $\text{NH}_4\text{-N}$ did not modify THC-COOH patterns.

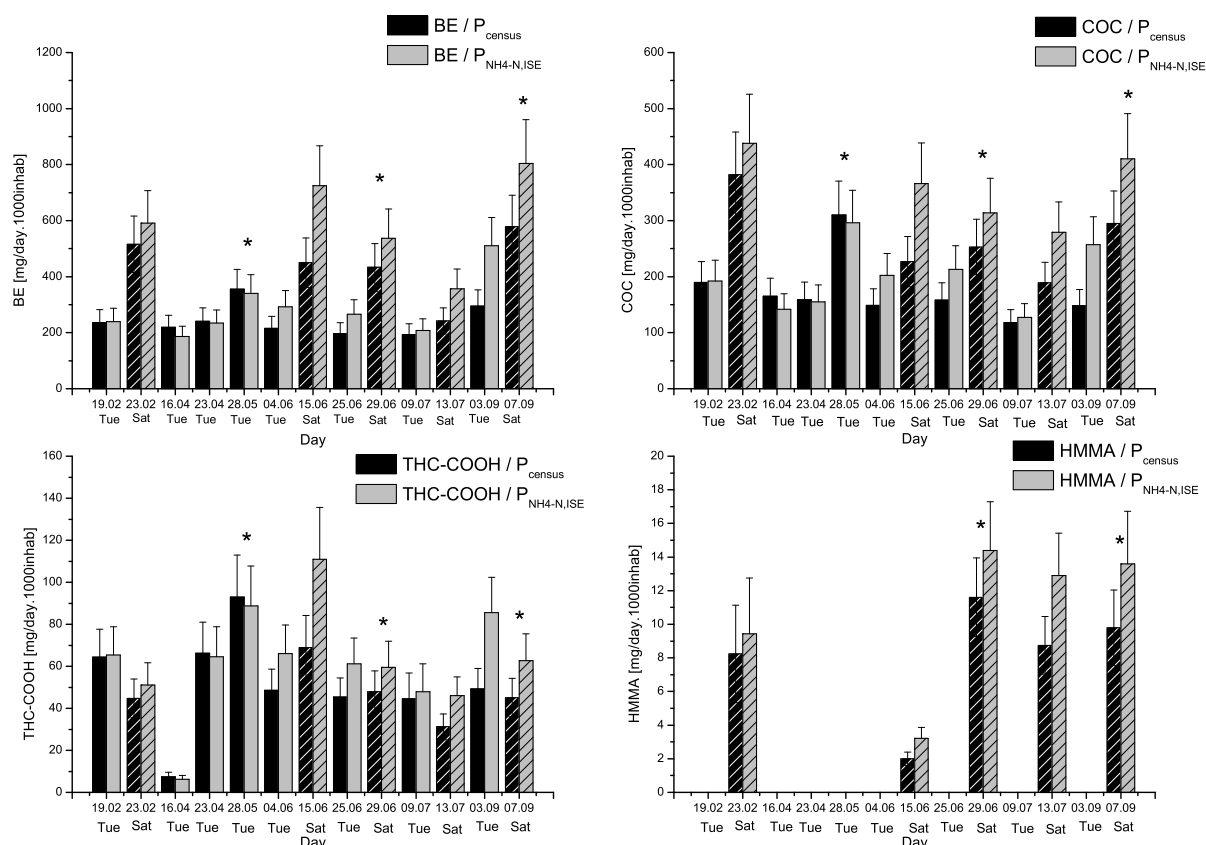


Figure 9: Per-capita loads of target drug residues (milligrams per day per 1000 inhabitants) based on census (i.e., 220'000 inhabitants, P_{census} shown in black) and NH₄-N estimates (P_{NH4-N,ISE} shown in grey). Striped bars show results for Saturdays-Sundays. Days with rain have been marked (*). Taken from Been et al. (76).

3.3 Major findings and conclusions

Targeted drug residues had a characteristic pattern during the weekend, which was attributed to the prolonged activity of people during weekend nights. Normalisation with NH₄-N resulted in an overall increase in the estimated per-capita loads, in particular during the weekends. The smaller non-resident population was considered as a potential explanation, however other unknown factors could be implicated. The major finding of this research was to demonstrate that normalising drug loads with a dynamic population marker such as NH₄-N, did not influence the pattern of drug loads. This could suggest that the variability in drug use is orders of magnitude higher compared to fluctuations in the size of the sampled population. Nonetheless, changes in the number of inhabitants could eventually occur over longer periods of time and, in particular for long-term studies, care should be taken to account for these. In this sense, measurement of NH₄-N could prove to be useful to detect eventual changes which would remain unnoticed using absolute loads or per capita loads based on static (e.g., census) indicators. Furthermore, this indicator has the advantage of being easily measurable.

IV Data triangulation in the context of opioids monitoring via wastewater analyses

This chapter is based on the following publication:

Been F., Benaglia L., Lucia S., Gervasoni J-P., Esseiva P., Delémont O., *Data Triangulation in the Context of Opioid Monitoring via Wastewater Analyses*, Drug and Alcohol Dependence, 2015, 151, 203-210; <http://dx.doi.org/10.1016/j.drugalcdep.2015.03.022>

For the complete publication, see *Annexe II*.

Wastewater-based epidemiology has been presented in the literature as an additional tool to monitor illicit drug consumption in a given community. Although providing quantitative data and performing comparative studies is highly relevant to assess the extent of illicit drug use (135,136), wastewater analysis has additional assets. Researchers and stakeholders have stressed the need to contextualise wastewater-based data and to combine it to epidemiological data (159,160), allowing to assess the relevance and complementarity of the indicators and to obtain a more informed picture of the phenomenon (159). Furthermore, there is a need to evaluate the reliability of the indicator by focusing on substances for which precise prescription or extensive epidemiological data is available.

The objectives of this part of the research are summarised in Figure 10. The first step consisted in testing the reliability of the methodology by comparing estimates of methadone consumption, derived from wastewater analyses, to figures obtained from the registry of people undergoing opioid substitution therapy, as well as from pain treatment prescriptions. The second objective consisted in evaluating if 6-monoacetylmorphine (an exclusive metabolite of heroin) and morphine loads could be used to estimate and monitor heroin consumption.

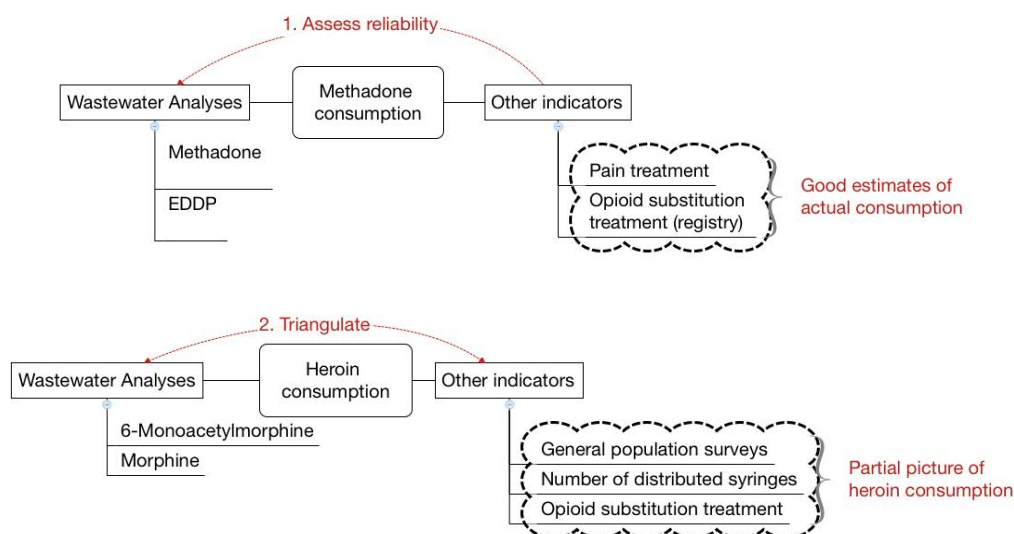


Figure 10: Scheme of the available data sources for methadone, heroin and morphine and the two objectives of the study. Adapted from Been et al. (85).

4.1 Study setup and available data

As for *Chapter III*, this study focussed on the metropolitan area of Lausanne. Samples were collected between October 2013 and July 2014, from Tuesdays to Wednesdays and from Saturdays to Sundays, 12 p.m. to 12 p.m., for a total of 28 samples. Prior to collection, sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) was added to the empty bottles of the autosampler to minimise bacterial activity (112). The autosampler was programmed to collect 65mL of wastewater every 5 minutes. Hourly samples (consisting of 12 x 65 mL of wastewater) were then mixed in a flow-proportional manner in the laboratory to obtain a 24 hours composite sample. The following substances were targeted: methadone and its major metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), the exclusive metabolite of heroin, 6-monoacetylmorphine (6-MAM), and morphine. Calculations and uncertainty estimates were computed using Monte Carlo simulations (for further details, see the publication in *Annexe II*). A summary of the parameters used is reported in Table 6.

Data sources about prescription of methadone in the catchment consisted of i) total methadone supplies for pharmacies, hospitals and physicians in Canton Vaud (no catchment specific data was available) between October 1st 2013 and June 30th 2014 (161) and ii) an anonymised registry of all patients undergoing opioid substitution therapy with methadone in Canton Vaud (162). Only patients living in the catchment area or patients whose physician's residence was within the catchment area were considered for this study.

Data sources to be compared to heroin consumption estimates derived from wastewater analysis were the following: i) the number of syringes distributed per month by specialised centres within the catchment area (163); ii) prevalence data about heroin consumption in Switzerland; iii) monthly morphine supplies in Canton Vaud between October 1st 2013 and June 30th 2014, provided by the authorities (161); iv) sales data about preparations containing other substances which are also metabolised to morphine (i.e., codeine, pholcodine, ethylmorphine and nicomorphine (35)).

Table 6: Parameters used for Monte Carlo simulations. a) Percentage of initial dose excreted as target compound; b) Ratio between the amounts of target compound adsorbed onto suspended matter and dissolved in the aqueous phase; c) Excretion after administration of heroin only; d) Excretion after administration of morphine only. SE = Standard Error. Taken from Been et al. (85).

	Source	μ	SE	Distribution	Note	
Flow	Initial data provided by the STP in $m^3 \cdot s^{-1}$ averaged over 5min (n = 288 measurements per day). Sum to obtain daily flow in $L \cdot day^{-1}$ from 12 p.m. to 12 p.m.	Daily flow [$L \cdot day^{-1}$]	Residuals from flow regression using a Gaussian model	Normal (μ, SE^2)	See Supporting Information of the publication for further details (Annexe II).	
Chemical Analysis	Result of chemical analysis and associated analytical error.	Mean concentration of 3 replicates [$ng \cdot L^{-1}$]	$\frac{SD}{\sqrt{3}}$	Normal (μ, SE^2)	Standard deviation derived from the validation of the analytical method and the analysis of 3 replicates	
Excretion ^a	(164)	EDDP: 25.0%	EDDP: 4.6%	Beta (a, b)	Data derived from the tables available in (164), i.e. 6 individuals submitted to 3 experiments (low, intermediate and high dosage)	
		MET: 19.7%	MET: 2.6%	Beta (a, b)		
	Data derived from literature review by Khan and Nicell (35)	6-MAM: 0.46%	6-MAM: 0.09%	Beta (a, b)		
		MOR ^c : 48.0%	MOR ^c : 2.4%	Beta (a, b)		Inverse-variance weighted average
		MOR ^d : 74.6%	MOR ^d : 1.6%	Beta (a, b)		
	6-MAM: 0.6%	6-MAM: 0.11%	Beta (a, b)	Inverse-variance weighted average of studies focused only on intravenous and intramuscular administration		
	MOR ^c : 53.4%	MOR ^c : 2.8%	Beta (a, b)			
Adsorption ^b	Used data from (114,165,166)	EDDP: 23.5%	EDDP: 5.9%	Normal (μ, SE^2)	Inverse-variance weighted average	
		MET: 12.1%	MET: 2.1%	Normal (μ, SE^2)		
	Used data from (165,166)	6-MAM: na	6-MAM: na	na	Inverse-variance weighted average	
		MOR: 4.1%	MOR: 1.1%	Normal (μ, SE^2)		

4.2 Calibration through the data related to methadone

Using the available registry and prescription data, it was possible to estimate that on average 67.7 grams of pure methadone were used per day in the investigated catchment. To obtain these figures, it was however necessary to assume that consumption across each month was homogeneous and that the available data sources are reliable.

Regarding wastewater measurements of methadone and its metabolite EDDP, an illustration of the measured loads is shown in Figure 11.

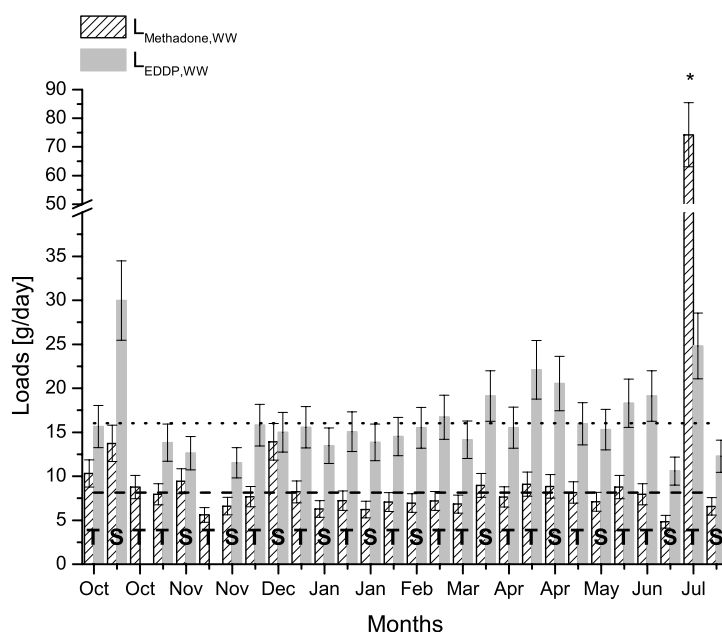


Figure 11: Methadone ($L_{\text{Methadone,WW}}$) and EDDP ($L_{\text{EDDP,WW}}$) loads measured during the sampling campaign. Figures reported here were not corrected for potential adsorption. The dotted and dashed lines represent the mean loads (after exclusion of the outliers) for EDDP and methadone, respectively. T = Sample collected from Tuesday to Wednesday, S = Sample collected from Saturday to Sunday. (*) $L_{\text{MET,WW}}$ outlier. Taken from Been et al. (85).

As can be seen, loads of both compounds appear to be stable throughout the sampling period, except for one peak in methadone loads which was identified as an outlier (Grubb’s test, $\alpha=0.05$). Interestingly, the measured methadone to EDDP ratio was in agreement with results from urine analysis of patients undergoing opioid substitution treatment (i.e., 1.8-2.2 (167,168)). Estimates of methadone consumption were computed via Monte Carlo simulations using both methadone and EDDP loads. Results are reported in Table 7.

Table 7: Mean methadone consumption estimated via Monte Carlo simulations with EDDP and methadone loads and estimates obtained from supplies data. Reported estimates were computed after exclusion of outliers. For results taking into account adsorption onto particulate matter, see the publication in *Annexe II*. Taken from Been et al. (85).

	<i>Methadone consumption [g/day]</i>		
	<i>Wastewater analyses</i>		<i>Supplies</i>
	<i>EDDP (n = 24)</i>	<i>Methadone (n = 26)</i>	
<i>Mean</i>	75.6	41.7	67.7
<i>SD</i>	19.3	8.7	-

When methadone was used, lower consumption estimates were obtained compared to supplies data. This could be due to the higher intra- and inter-individual excretion variability of methadone (167–169), which could translate in a less accurate estimate of the true excretion rate. The good agreement between estimates obtained with EDDP and registry/prescription data support previous findings that alternative sources of methadone are limited in this context

(170). Most importantly, however, these results show that the sampling scheme used allows to collect representative samples, thus enabling to monitor the consumption of a small group of individuals. Therefore, it should be possible to efficiently monitor the consumption of similar, eventually smaller (e.g., heroin users), or supposedly larger groups of drug users (e.g., cannabis and cocaine users). Furthermore, these findings suggest that, for methadone and its metabolite, losses and degradation in sewers are negligible.

4.3 Monitoring heroin use

Through the needle and syringe distribution program carried out in the city of Lausanne, it was possible to measure the number of syringes distributed each month (i.e., average of 12'508 \pm 1339 per month). The following assumptions were made to estimate heroin consumption based on these figures: i) syringes are distributed homogeneously across each month and ii) are used only once; iii) only 52.6% of syringes are used to inject heroin (according to reports from injecting drug users (IDUs) visiting low threshold facilities in Switzerland (171)) and iv) a dose ranges from 30 to 100 mg of pure heroin (145,172), with a midpoint at 65mg. Hence, daily consumption is estimated between 6.5 \pm 0.6 and 21.6 \pm 2.0 g/day (midpoint 14.1 \pm 1.3 g/day) of pure heroin, depending on the considered dosage. Similar figures were obtained with survey data (i.e., prevalence). In Switzerland, 12-months prevalence of heroin use in the population aged 15 to 75 was estimated to 0.1% (173). Transposed to Lausanne's metropolitan area, this corresponds to 193 potential heroin users which, assuming they consume one dose per day (30, 65 or 100 mg/dose, as previously), would give a daily consumption between 5.8-19.3 g/day of pure heroin.

The results from the wastewater monitoring campaign are reported in Figure 12. In the case of 6-MAM, no particular trend could be observed in the data, except for three peaks identified as outliers (Grubbs Test, $\alpha = 0.05$), which could potentially be due to direct disposal of heroin in sewers, although this remains only a speculation. In the case of morphine loads, a nearly two-fold increase was observed after the month of February and the reason for this observation remains unknown.

Monte Carlo simulations were used to estimate the amount of heroin consumed based on measured 6-MAM and morphine loads and parameters reported in Table 6. As can be seen from results reported in Table 8, figures obtained with 6-MAM are inconsistent. In fact, if one considers that the average purity of street heroin seizures is 11% (174), this would correspond to a consumption of 4.3 kg/day of heroin, which, for a population of only 226'000 inhabitants, is unreasonably high. This is even clearer when observing estimates derived from morphine which, although being an overestimation because of the contribution of legal sources of morphine (i.e., prescriptions), are considerably lower (i.e., 51.6 \pm 8.9 g/day or approximately 479 g/day at street purity).

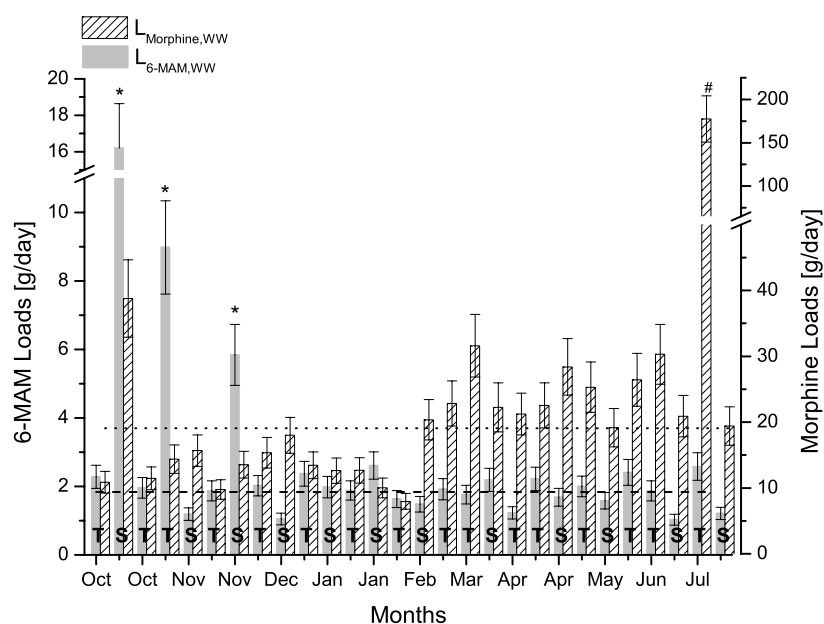


Figure 12: Morphine ($L_{\text{Morphine,WW}}$) and 6-monoacetylmorphine ($L_{\text{6-MAM,WW}}$) loads not corrected for adsorption, measured during the sampling campaign. The dotted and dashed lines represent the mean loads (after exclusion of the outliers) for $L_{\text{Morphine,WW}}$ and $L_{\text{6-MAM,WW}}$, respectively. T = Sample collected from Tuesday to Wednesday, S = Sample collected from Saturday to Sunday. (#) $L_{\text{Morphine,WW}}$ outlier, (*) $L_{\text{6-MAM,WW}}$ outliers. Taken from Been et al. (85).

Table 8: Amount of pure heroin consumed in the catchment estimated from 6-MAM and morphine loads using Monte Carlo simulations. Reported estimates were computed after exclusion of outliers. For results taking into account adsorption onto particulate matter, see the publication in *Annexe II*. Taken from Been et al. (85).

	Heroin [g/day]	
	6-MAM (n=24)	Morphine (n=26)
Mean	542.1	51.6
SD	163.4	8.9

If estimates of heroin consumption are made based on 6-MAM loads reported in other studies, inconsistent figures are obtained with regard to the size of the considered population and the heroin prevalence reported in these areas. These inconsistencies could be due to i) the limited number of clinical trials having studied the excretion of heroin (116); ii) the low stability of 6-MAM in wastewater (86,114,121,123); iii) the different excretion rates depending on the administration route; iv) the contribution from faecal excretion; v) the proportion of 6-MAM already present in street heroin (175,176), a fraction of which could eventually be excreted unchanged and thus contribute to measured loads.

Using morphine to back-calculate heroin consumption implicates that the fraction of morphine generated from the (legal) use of pharmaceuticals (i.e., morphine, codeine, pholcodine, ethylmorphine and nicomorphine (35)) has to be subtracted from the measured loads. Prescription and sell-in data about these pharmaceuticals for the sampling period (161,177) were used to calculate the difference between measured and expected morphine loads. Because morphine prescription data were representative of the entire region (i.e., Canton Vaud), estimates for the catchment area were computed based on i) the proportion of people

living in the latter and ii) the number of hospital beds. The midpoint between the two estimates was taken for the subsequent calculations. Similarly, the number of inhabitants and hospital beds were used to estimate morphine loads generated from consumption of codeine, pholcodine and ethylmorphine (data about nicomorphine was not available). Loads generated from all prescription pharmaceuticals were summed and subtracted from measured loads. For these calculations, it was assumed that stocks and disposal of unused medications are negligible and that all prescribed pharmaceuticals are consumed. Results are shown in Figure 13.

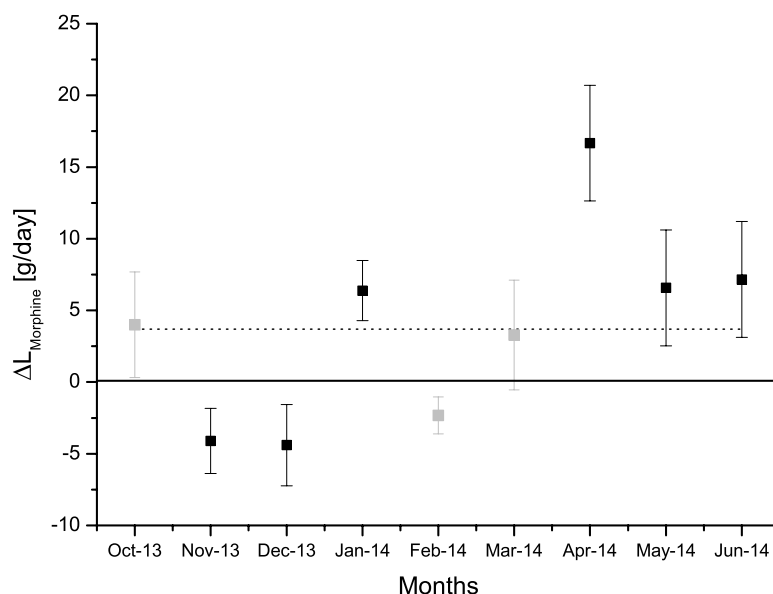


Figure 13: Difference ($\Delta L_{\text{Morphine}}$) between measured and expected morphine loads. For each month, average measured morphine loads were calculated and subtracted to the expected loads generated from pharmaceuticals for the corresponding month. Non-significant differences are shown in grey. The dotted line represents the average $\Delta L_{\text{Morphine}}$. Taken from Been et al. (85)

For three months, the difference between measured and expected loads was not significant (t-Test, p -value > 0.05, see Figure 13). Negative net morphine loads were obtained when measured loads were below prescription/sales figures. This emphasises the need to collect an adequate number of samples, without which the net difference between measured and expected loads cannot be reliably assessed. The mean difference between measured and expected loads and the corresponding heroin consumption are reported in Table 9.

Table 9: The first part of the table reports morphine loads measured in wastewater and expected from consumption of pharmaceuticals. The difference was calculated by subtracting expected from measured loads. The second part of the table reports the estimated heroin consumption back-calculated from the net difference (which is assumed to be excreted after heroin consumption), from syringe distribution data and general population surveys (using three dosages). a) The mean reported here was calculated from the average morphine loads computed for each month. The standard deviation was calculated as the square root of the of the average variance calculated over all months. Taken from Been et al. (85)

<i>Morphine loads [g/day] (SD)</i>		
<i>^aMeasured in wastewater</i>		19.1 (3.1)
<i>Expected from sales and prescription</i>		15.0 (0.5)
<i>Measured in wastewater – Expected</i>		4.7 (6.5)
<i>Estimated heroin consumption [g/day] (SD)</i>		
<i>Measured – Expected</i>		12.7 (8.3)
	<i>Dose [mg]</i>	
<i>Syringes</i>	30	6.5
	65	14.1
	100	21.6
<i>Survey</i>	30	5.8
	65	12.5
	100	19.3

Based on wastewater measurements and prescription/sales data, it was estimated that 12.7 grams of pure heroin are consumed on average per day. This corresponds, at street level purity (i.e., 11%), to 115.5 g/day. These results are in agreement with estimates derived from the other available data sources (i.e., number of distributed syringes and population survey).

4.4 Major findings and conclusions

Using a detailed registry about methadone distribution in the investigated area allowed to partially validate the methodology implemented in the context of this study. In particular, the sampling design was shown suitable to collect representative wastewater samples to assess the consumption of a small cohort. These results suggest that the methodology can be extended to monitor the consumption of other groups of drug users.

In the case of heroin, various assumptions were made initially, based on which consumption estimates were computed. Nonetheless, the good agreement between the indicators allows making some inferences about heroin consumption in the investigated area. Firstly, the findings support the initial hypothesis that injection is not the unique administration route. According to survey data, 18.4% and 26.3% reported fumigation and nasal insufflation as major administration route (171). Summed, these two groups make up almost 45% of questioned heroin users. If it is assumed that these two groups are also responsible for consumption of almost half the estimated daily consumption, then a dosage of 30mg/dose for injection appears to be the most likely. In fact, if this is taken, then the daily consumption due to IDUs would correspond to 6.5g/day of pure heroin (see Table 9), leaving 6.2 g/day which could potentially be due to users preferring fumigation and/or nasal insufflation. Estimates

derived from survey data are in agreement with wastewater measurements, in particular when 65mg/dose is used as base for the calculations. When combined, the results seem to suggest that the actual heroin dosage lies somewhere between 30 and 65 mg (fumigation and insufflation requiring higher dosages because of lower bioavailability (178)). Furthermore, daily consumption and the estimated number of heroin users (i.e., 193) seem to be indicative of heroin usage in the investigated area.

V Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland

This chapter is based on the following work, which has been submitted for publication:

Been F., Bijlsma L., Benaglia L., Berset J-D., Botero-Coy A. M., Castiglioni S., Kraus L., Zobel L., Schaub M., Bücheli A., Hernández F., Delémont O., Esseiva P., Ort C.; *Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland. submitted.*

For the complete publication, see *Annexe III*.

Wastewater-based epidemiology has some clear advantages compared to more conventional approaches used to assess and monitor the extent of drug use at the regional and national scale. Among the issues encountered with conventional methods, one can cite reporting bias and concealment, overrepresentation of visible drug users, long study times and costs (136,179). Nonetheless, highly important pieces of information such as age, sex, social and economical background or history of drug use, can only be gathered by conventional methods. Epidemiologists and addiction researchers are thus faced with the problem of trying to measure a hidden phenomenon, using a set of indicators which will only provide a partial picture of the situation. For this reason, the combination of multiple indicators will likely help overcoming these limitations and provide a more precise picture of the phenomenon, both at the regional and national scale.

The work presented here is the results of a collaboration between different actors, from five European countries, involved in wastewater-based epidemiology, addiction research and drug epidemiology.

The objective of this work consisted in investigating geographical differences in illicit drug use in Germany and Switzerland (and Liechtenstein, although it was considered as an adjacent region of Switzerland) identified through a set of complementary indicators: i) survey data, ii) consumption offences reported by law enforcement and iii) wastewater analysis. The indicators were compared to determine the extent of overlap and hypotheses were formulated, in an attempt to explain observed discrepancies and further understand how wastewater analyses could help overcoming some of the limitations encountered in this context. Finally, to obtain a rough idea of the size of the drug market, local and nation-wide consumption estimates were computed. For this, measured loads and Monte Carlo methods were used to estimate daily and weekly consumption in the cities included in the sampling campaign. Then,

using a regression model including the number of inhabitants, local figures were used to extrapolate nation-wide consumption estimates.

5.1 Target cities and available data sources

Target of the present study were five cities in Germany (Munich, Dresden, Berlin, Dortmund and Dülmen), 13 cities in Switzerland (Geneva, Lausanne, Neuchâtel, Sion, Biel, Berne, Basel, Lucerne, Zurich, Winterthur, St. Gallen, Chur and Lugano) and one city in Liechtenstein (Bendern). Wastewater samples were collected in the corresponding WWTP during one week in March 2014. It should be noticed that the catchments generally go beyond the political boundaries of the investigated cities and also include their suburbs. In the particular case of Berlin, samples were collected in the four WWTP serving the city. Parameters used for the Monte Carlo simulations are reported in Table 10 and Table 11.

Twelve-months prevalence data for Germany and Switzerland (no data was available for Liechtenstein) were obtained from general population surveys (GPS) conducted in the investigated areas (9,180–184). Information about usage of the following substances was included in the questionnaires: cocaine, cannabis, amphetamine-type stimulants (ATS, for Germany only) – such as amphetamine, methamphetamine and MDMA – and heroin (for Switzerland only). Yearly consumption offences were provided by local police forces.

Data were presented as maps, allowing for a direct comparison of the considered indicators, as shown in Figure 14 and Figure 15.

Table 10: General parameters used in Monte Carlo simulations to estimate daily mass loads [g/day] of illicit drugs and their metabolites. μ = mean, SE = standard error. Taken from Been et al. (185).

<i>Parameter</i>	μ	<i>SE</i>	<i>Distribution</i>	<i>Note</i>
<i>Flow</i>	Daily flow [L/day]	20% of daily flow	Normal (μ , SE ²)	Based on findings by Ort et al. (95,100), the error associated to daily flow measurements (S.E.) was estimated as 20% of the total daily flow
<i>Substance concentration</i>	Measured concentration [ng/L]	25% of measured concentration	Normal (μ , SE ²)	From a preliminary evaluation of an inter-laboratory test, an average deviation of 25% from the expected values was reported by the participating laboratories.

Table 11: Compound specific parameters used in Monte Carlo simulations to back-calculate the amounts of parent compound initially consumed. μ = mean, SE = standard error. Taken from Been et al. (185).

	<i>Substance</i>	μ	<i>SE</i>	<i>Distribution</i>	<i>Note</i>
<i>Excretion rates</i>	Benzoyllecgonine	30.58%	3.35%	Beta (a,b)	Inverse-variance weighted average. Data derived from summary in (35,186)
	MDMA	15.78%	1.83%		
	Amphetamine	29.12%	0.93%		
	Methamphetamine	28.56%	2.59%		

Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland

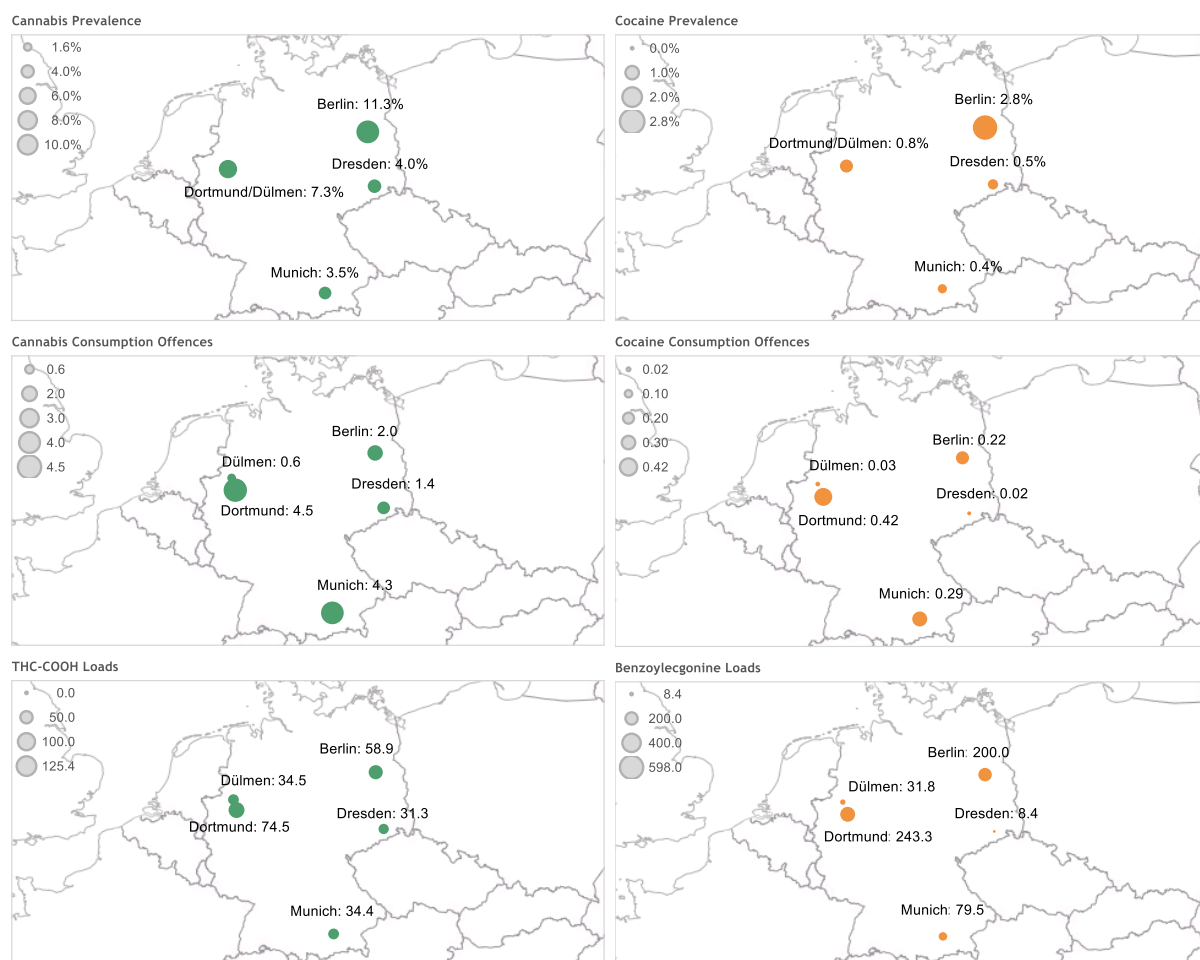


Figure 14: 12-months prevalence for cocaine and cannabis in Berlin and the federal states of Northrhine-Westfalia (Dortmund and Dülmen), Saxony (Dresden) and Bavaria (Munich). Reported consumption offences per year per thousand inhabitants. Average population normalised loads [$\text{mg}\cdot\text{day}^{-1}\cdot 1000\text{inhab}^{-1}$]. Only data from three of the four WWTP sampled in Berlin are reported (weekend data were missing for Berlin-Münchehofe). Taken from Been et al. (185).

Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland

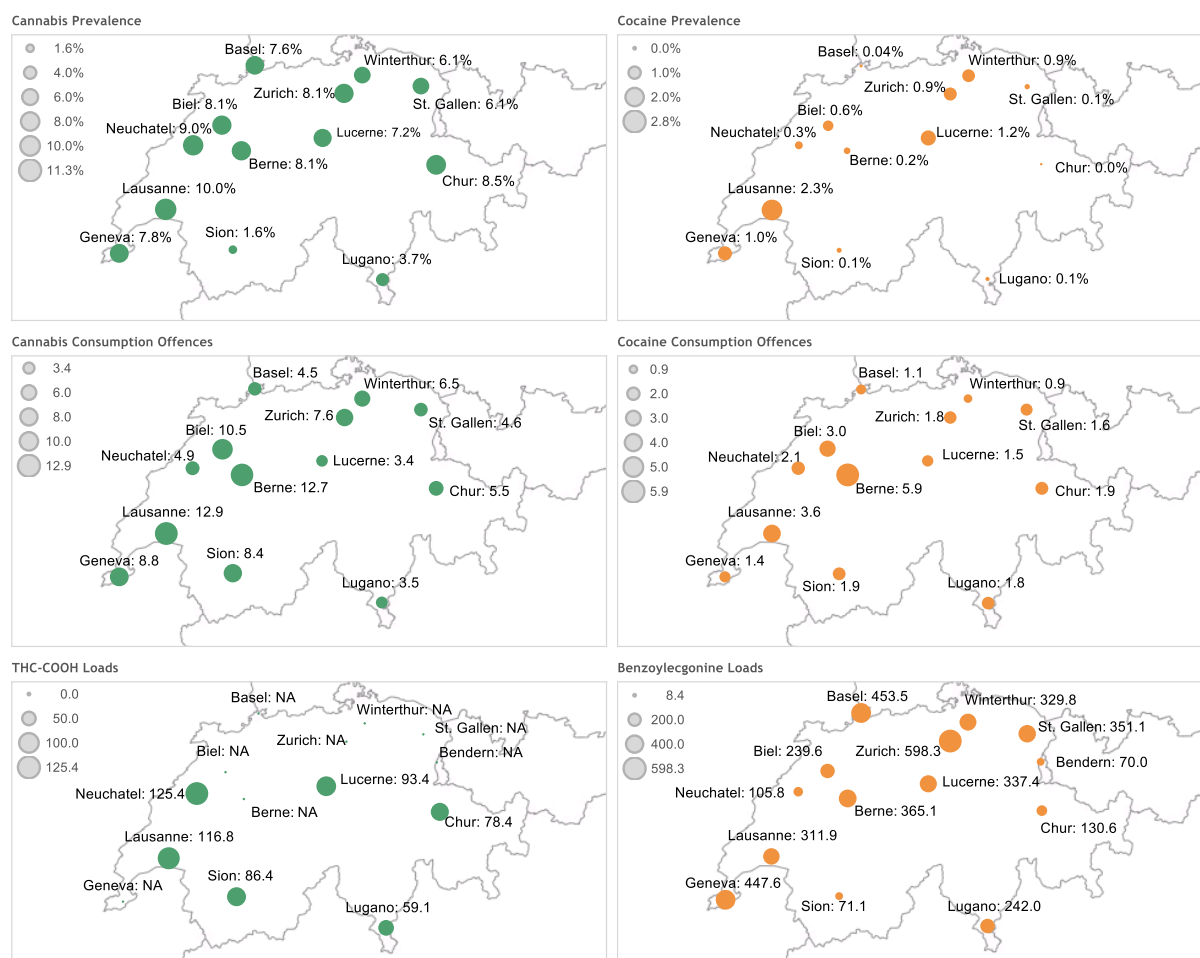


Figure 15: 12-months prevalence, reported consumption offences per year per thousand inhabitants and average population normalized loads [mg.day⁻¹.1000 inhabitants⁻¹], for cocaine and cannabis in Switzerland and Lichtenstein (wastewater data only). Wastewater samples from Geneva, Bern, Biel, Basel, Winterthur, Zurich and Bendern, were not analysed for THC-COOH. Taken from Been et al. (185).

Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland

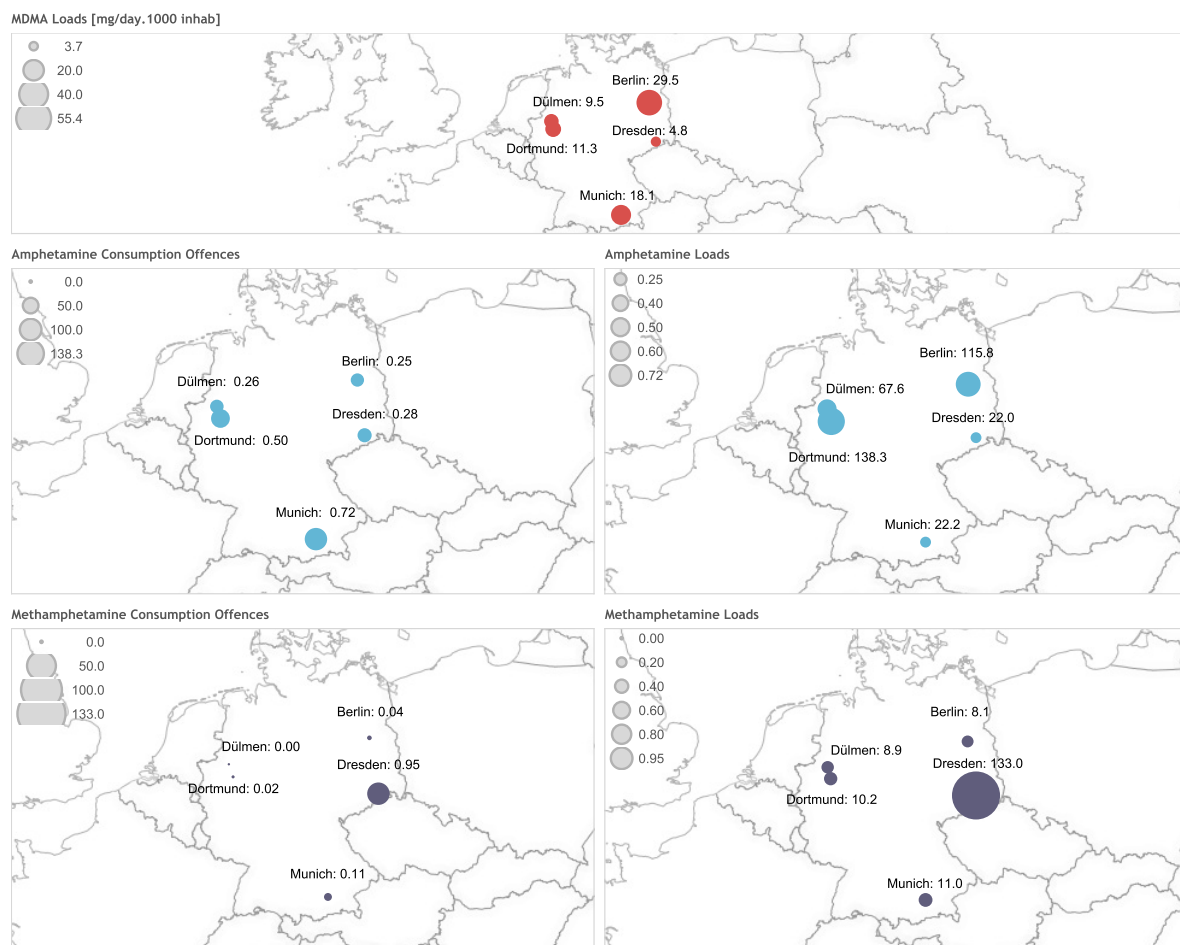


Figure 16: Average population normalised loads [mg.day⁻¹.1000 inhabitants⁻¹] for MDMA, amphetamine and methamphetamine measured in Germany. Reported offences per year per thousand inhabitants in Germany. Offences data for MDMA was not available. Taken from Been et al. (185).

Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland

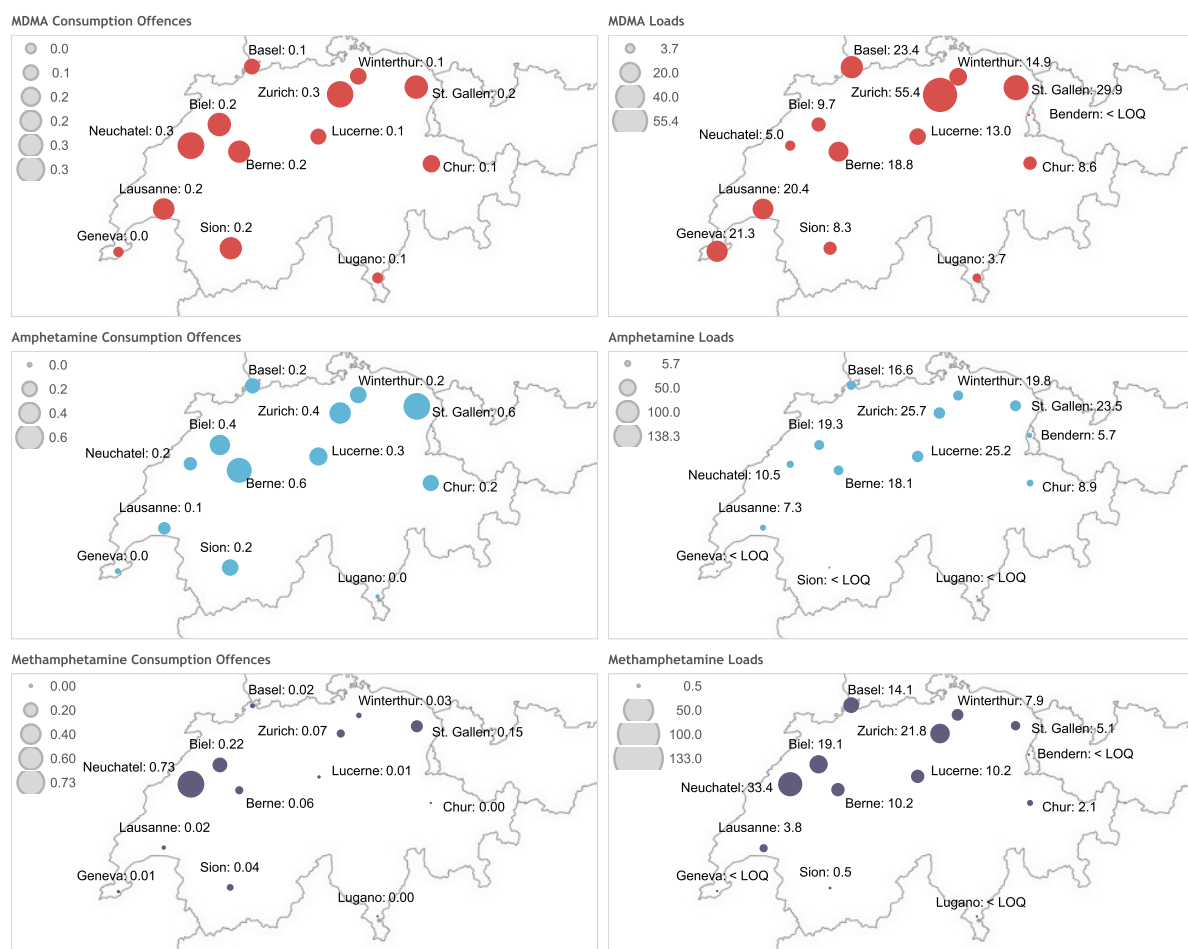


Figure 17: Reported consumption offences (per year per thousand inhabitants) and average population normalised loads [mg.day⁻¹.1000 inhabitants⁻¹] for MDMA, amphetamine and methamphetamine measured in Switzerland and Liechtenstein. Taken from Been et al. (185).

5.2 Comparison of epidemiological, police and wastewater data

In the case of Germany, an overlap between available data sources about cocaine consumption was observed, although some minor discrepancies still existed. This was not the case for Switzerland, where each data source drew a distinct picture. According to wastewater, per capita loads increased as a function of the population, suggesting that cocaine use is positively correlated to the size of the investigated population. For cannabis, prevalence and wastewater data allowed to draw a very similar picture in both Germany and Switzerland. On the contrary, offences showed a more heterogeneous image of cannabis consumption in both countries. In the case of ATS, wastewater data confirmed the widespread use of methamphetamine in the eastern city of Dresden (see Figure 16). But it also suggested that consumption is limited to this area, although further cities should be sampled to obtain a more precise cartography of methamphetamine use in the eastern part of the country.

According to wastewater data, amphetamine is the major stimulant drug, particularly in northern cities of Germany. In Switzerland, wastewater analysis allowed to highlight the more widespread use of amphetamine in the north-eastern parts of the country (see Figure 17). Furthermore, it confirmed the existence of a hotspot of methamphetamine usage around the cities of Neuchâtel, Biel and Bern.

Using the collected wastewater data about benzoylecgonine and MDMA, nationwide consumption estimates for Switzerland were computed. For cocaine, mixed effect models allowed to estimate a daily and weekly consumption of 8.8 and 61.6 kg of pure substance, respectively. For MDMA, ordinary least square regressions had to be used and the average daily consumption was estimated to 367 g of pure substance.

5.3 Major findings and conclusions

The results of this study show that, when trying to investigate geographical differences in drug use, substantially different pictures are drawn when conventional indicators, such as surveys and crime statistics, are considered. In the specific case of surveys, discrepancies from wastewater data can be due to reporting biases but also to differential perception (or stigmatisation) of drug use. However, this seems to affect mainly cocaine and heroin. For the other substances, included ATS, these biases are clearly less pronounced, both in Germany and Switzerland. Offence rates are, to a certain extent, influenced by law enforcement strategies, themselves influenced by public opinion (e.g., visibility of the drug scene) and politics, hence affecting their representatives of actual drug use. In this context, wastewater analysis has been shown to be a useful tool to overcome these limitations. However, as already highlighted, it is not a “specific” method, in the sense that it will only provide information about consumption, not about the consumers. As such, it should be combined to other sources of data to get the whole picture. Nonetheless, in the context of this work, wastewater analysis allowed to determine that cannabis was ubiquitously and homogeneously consumed in the investigated areas. Furthermore, the overlap between indicators of ATS use suggests that these substances have a less negative image, but also that their consumption remains predominantly occasional/recreational. This implicates that there is a limited number of regular/heavy users, which conventional indicators would have likely failed to capture.

At the hand of the results obtained here, some hypotheses about regional futures could be confirmed. However, because existing methods did not allow to reach such a level of detail, some previously unknown particularities could be identified (e.g., more widespread use of amphetamine in north-eastern Switzerland compared to the western part). Nonetheless, even if wastewater analysis has some clear advantages compared to conventional methods, these results should still be interpreted carefully. Ideally, the findings should be strengthened by performing longer and regular sampling campaigns. Furthermore, when comparing different locations based on results from wastewater analysis, care should be taken to consider the potential influences which are due to factors other than the structure of drug use. Namely, the different sampling modes, flow measurements, sewer architectures and analyte stability. A detailed discussion of these aspects will be presented in *Section 9.2*.

VI Integrating environmental and self-report data to refine cannabis prevalence estimates in a major urban area of Switzerland

This chapter is based on the following work, which has been submitted for publication:

Been F., Schneider C., Zobel F., Delémont O., Esseiva P.; *Integrating environmental and self-report data to refine cannabis prevalence estimates in a major urban area of Switzerland, submitted.*

For the complete publication, see *Annexe IV*.

11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH), an exclusive metabolite of the major of delta-9-tetrahydrocannabinol (THC), the active compound found in cannabis, has been monitored since the first applications of WBE. However, the challenges faced with the analysis of THC-COOH and the scarce pharmacokinetic literature have limited its extensive use to monitor cannabis use and estimate consumption. Despite this difficulty, monitoring cannabis is highly relevant at present due to the widespread discussions about legalising its use and regulating the market (187,188).

The objective of this part of the research was to merge epidemiological and wastewater data about cannabis consumption into a single inference model in order to refine current estimates about prevalence, overcome some of the weaknesses affecting the two indicators and evaluate the added value of WBE in respect of conventional indicators. Epidemiological data consisted of surveys conducted in Switzerland and Europe aiming at identifying, based on frequency of use, the different categories of users and their average consumption. Wastewater data consisted of samples collected between October 2013 and December 2014 at the WWTP of Lausanne, analysed to determine THC-COOH concentrations and calculate daily loads. Furthermore, available data about urinary and faecal excretion of THC-COOH were collected and used to estimate the total excretion.

6.1 Modelling

The collected data sources were combined in a Bayesian hierarchical model, as shown in Figure 18. The model consisted of three sub-models. The first part, referred to as *Wastewater* in Figure 18, was inspired by previous work by Jones et al. (147) and was used to estimate the amounts of pure THC consumed, based on THC-COOH loads measured in wastewater. For details about the various parameters used, see Table 40 in *Annexe IV*. The part named *Prevalence* was used to model the yearly prevalence according to results from a survey conducted in Switzerland in 2013 (173). For the population aged 15 or older living in western Switzerland, this was estimated to 6.2%. The part named *User types* was used to model the different types of users, classified into four categories, based on the number of days of use per months reported in the abovementioned survey (173). Unfortunately, no information was

available about the amounts of cannabis consumed by each category of users in Switzerland. However, in an extensive study by van Laar et al. (189), the average consumption of the different typologies of cannabis users was investigated in various European countries. Obtained figures, in particular those gathered for Italy, the Netherlands and Portugal, were used as reference values for Switzerland. These countries were chosen because consumption habits are assumed to be the most similar to Switzerland. The sub-models described in *Prevalence* and *User types* were used to estimate the number of users and the daily consumption of pure THC, based on epidemiological information. The considered figures were used as initial values (i.e., priors) for the full model including also wastewater data. To translate pure THC estimates into amounts of cannabis, the average purity of seizures analysed in laboratories across Switzerland was used (i.e., approximately 11% (190)).

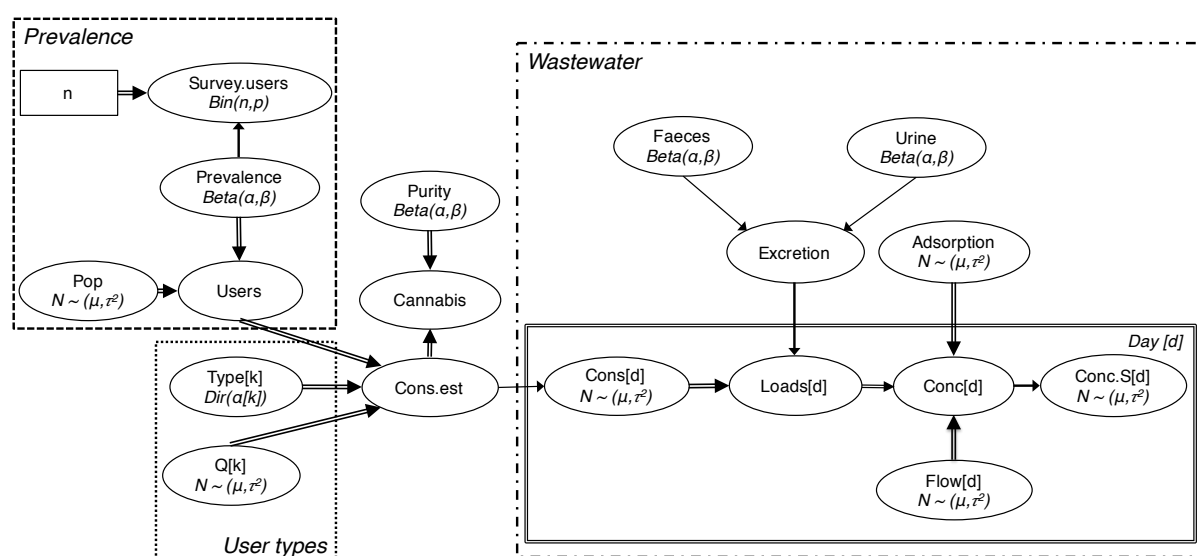


Figure 18: Directed acyclic graph illustrating the hierarchical model used to estimate prevalence of cannabis consumption in the investigated area. Nodes represent stochastic variables (round) and constants (squares). Arrows represent stochastic (single) and logical (double) relationships. *Prevalence*: refers to the modelling of prevalence of cannabis consumption in the investigated area; *User types*: refers to the modelling of user types and their daily consumption; *Wastewater*: refers to modelling of excretion, occurrence in wastewater and analysis of THC-COOH and was founded on previous work by Jones et al. (147). Taken from Been et al. (36).

6.2 Temporal data

Temporal data of THC-COOH loads measured between October 2013 and December 2014 is shown in Figure 19. The absence of a trend is particularly interesting with respect to the recent changes in drug policies which took place in Switzerland. In fact, since the 1st of October 2013, consumption of cannabis has been decriminalised and is now only punished by an administrative fine (191). Although the sampling campaign started only on the 22nd of October 2013, it can reasonably be inferred that, at least during 2014, consumption of cannabis has not increased. From a drug policy perspective, this is a relevant information as it suggests that changes in regulation did not promote consumption.

Integrating environmental and self-report data to refine cannabis prevalence estimates in a major urban area of Switzerland

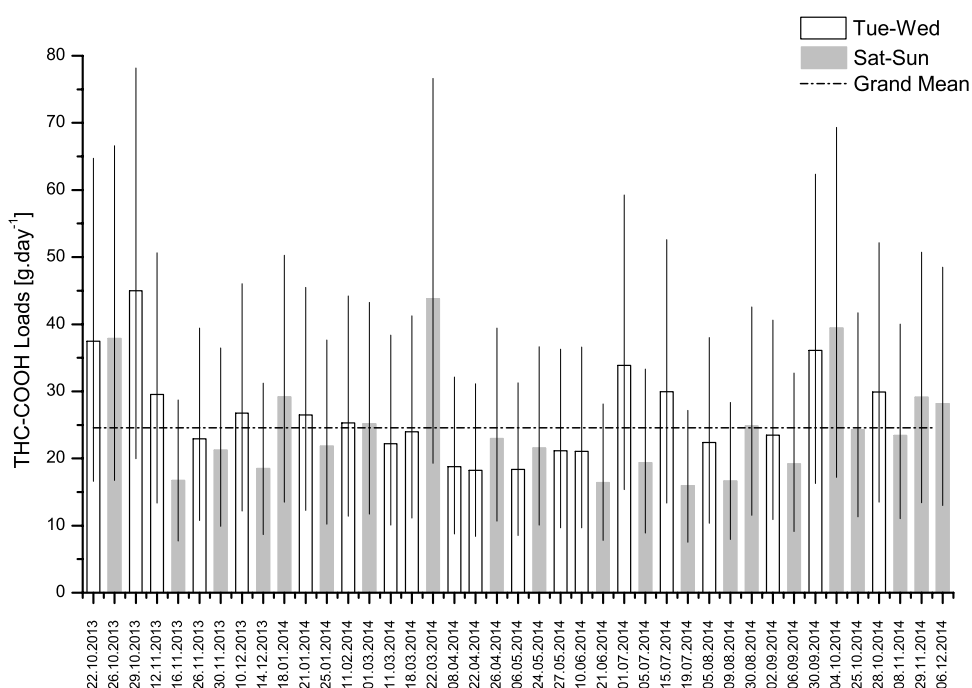


Figure 19: Estimated THC-COOH loads over the sampling period. Samples collected between Tuesday-Wednesday and Saturday-Sunday are shown in white and grey, respectively. The vertical lines show the upper and lower 95% credible intervals (CI). The dashed line shows the average loads.

Table 12: Results for estimation of the various parameters linked to cannabis consumption using the model described in Figure 18. The number of users and prevalence refer to past year consumption.

CI = credible interval. a) Results derived from survey data (173).

<i>Model</i>		<i>Average daily THC consumption [g.day⁻¹]</i>	<i>Average daily cannabis consumption [kg.day⁻¹]</i>	<i>Number of users</i>	<i>Prevalence</i>
Wastewater data only	<i>Estimate</i>	352.1	3.8	-	-
	<i>95% CI</i>	126.7 - 1290.8	1.1 - 11.5	-	-
Epidemiological data only	<i>Estimate</i>	182.8	1.6	10520	^a 6.2%
	<i>95% CI</i>	85.2-312.1	0.7-2.8	6'277-15'000	5.3-7.1%
Epidemiological and wastewater data	<i>Estimate</i>	321.7	2.9	17090	9.5%
	<i>95% CI</i>	191.5-493.1	1.7-4.4	11'490-23'250	8.4-10.6%

6.3 Prevalence estimation

The results from the modelling of consumption and prevalence are reported in Table 12. Using only the model containing information about wastewater analysis and excretion of THC-COOH, it was estimated that 352 g/day of pure THC are consumed on average, corresponding to an estimated 3.8 kg/day of cannabis. As can be seen, however, large credible intervals were obtained. Estimates obtained using only epidemiological data were substantially lower. Using the complete model, integrating both epidemiological and wastewater data, the average daily consumption was estimated to 321.7 g/day of pure THC, or 2.9 kg/day in terms of cannabis. Interestingly, the credible intervals could be significantly reduced. The updated estimate of prevalence was substantially higher compared to survey figures (i.e., 9.5% *versus* 6.2%), as can be seen on Figure 20. These were also higher compared to nation-wide estimates for urbanised areas, which have been shown to have higher prevalence (i.e., 7.5% (173)). If the proportions of users falling in each category are considered representative of the investigated catchment then, out of the 17'090 people who consumed cannabis at least once a year, approximately 7400 would be infrequent users (i.e., using cannabis between 1 and 3 days per month), 4204 occasional (i.e., 4-9 days per month), 2307 regular (i.e., 10-19 days per month) and 3179 intensive (i.e., more than 20 days per month). Approximately 97% of the total consumption would be due to the latter two groups of more serious consumers. Yet, because also with cannabis, heavy users are likely difficult to measure via surveys (e.g., underreporting), these figures should be interpreted carefully. Nonetheless, the obtained estimate is likely closer to the true cannabis prevalence compared to figures derived from survey data only.

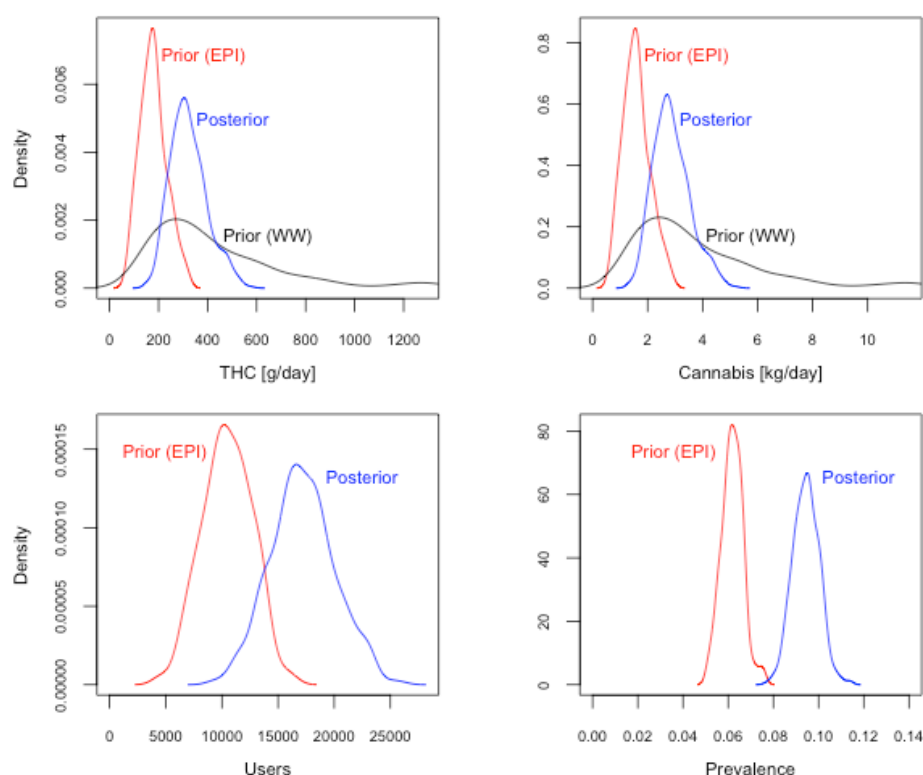


Figure 20: Outputs of the Markov Chain Monte Carlo simulations using epidemiological (Prior (EPI), red curves), wastewater (Prior (WW), black curve) and the combination of epidemiological and wastewater data (Posterior, blue curve).

6.4 Major findings and conclusions

Globally, including figures from wastewater analysis resulted in an higher estimate of both consumption and prevalence compared to survey data (between 26 and 58% higher). Interestingly, the obtained difference corresponds to what is commonly found when self-reports of alcohol consumption are compared to sales data (192,193). Underreporting, recall bias and inaccurate estimate of past intake, which have often been called into question to explain differences in the field of alcohol, are likely applicable also to the context of cannabis use. However, these results could have not been obtained without the integration of data derived from approaches other than WBE. Information about the structure of cannabis or use (i.e., user types, consumption habits and frequency of use), or that of any other type of substance, will be available only through more direct methods whose focus are consumers and not, as it is the case for WBE, consumption. Furthermore, the uncertainties which still affect the measurement of consumption via wastewater analysis should not be disregarded. The approach presented here illustrates the methodological frame and the advantage of combining complementary sources of data to obtain refined estimates of drug use. Although the obtained figures are still affected by various sources of uncertainty and bias (i.e., underreporting of surveys, instability of THC-COOH and scarce pharmacokinetic data), they can be used to assess the potential prevalence and consumption of cannabis in an urbanised

area in Switzerland. With regard to the known issue that surveys will underestimate cannabis use, these figures provide quantitative evidence of what the true prevalence could be. Whilst still imperfect, wastewater analysis adds a crucial piece to the puzzle and allows to improve current knowledge about cannabis use. Furthermore, the presented approach could be extended to i) understand how the various typologies of users, and their respective consumption, would affect estimates of consumption, ii) include additional information from other epidemiological sources and iii) model the consumption of other substances. These aspects will be further discussed in *Section 9.3*.

VII Wastewater-based epidemiology from the perspective of law enforcement

Part of this chapter is based on the following publication:

Been F., Schneider C., Delémont O., Esseiva P.; *Können Abwasseranalysen der Polizei helfen, Drogenmärkte besser zu verstehen?*, *Kriminalistik*, 2015, 11, 628-634.

For the complete publication, see *Annexe V*.

In the literature, wastewater-based epidemiology has been presented as a beneficial addition to current methods used to monitor illicit drug use. Yet, its utility from the perspective of law enforcement has rarely been addressed or formalised. So one might ask, but are estimates derived from the analysis of wastewater samples useful for the police? Or do consumption estimates merely nourish the prejudice that their work has little impact and that the vision they have of drug use is only reflect of their activity? However, the work of law enforcement goes beyond simply arresting dealers and seizing drugs, and both its activity and vision about the drug market can be useful to understand the epidemiology of drug use (194). It is in this context that wastewater analysis could potentially be used to help drug enforcement in their activities, and vice versa, the information gathered by the police could help better understand the results of wastewater analysis.

The objective of this part of the research consisted in evaluating the added value of WBE from a drug enforcement perspective. In particular, emphasis was set on determining if and how the results from wastewater analysis could be used to better understand the structure of the drug market in the cities of Lausanne and Neuchâtel, in western Switzerland. Focus was set on cocaine, heroin and methamphetamine.

7.1 Cocaine

Results presented in Figure 21 consist of 42 wastewater samples collected at the influent of the WWTP of Lausanne and its suburbs between October 2013 and December 2014. As for *Chapters IV* and *VI*, samples were collected from Tuesday to Wednesday and from Saturday to Sunday, 12 p.m. to 12 p.m..

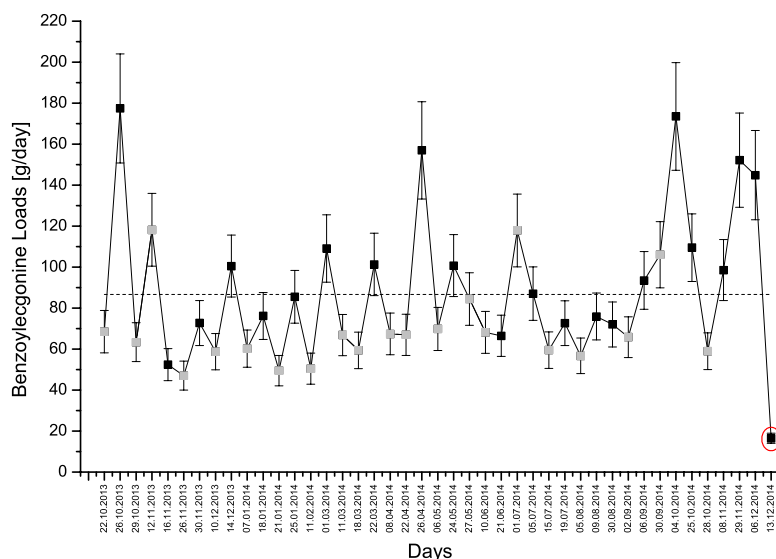


Figure 21: Estimated benzoylecgonine loads in Lausanne between October 2013 and December 2014. Samples collected between Tuesday and Wednesday are shown in grey. Samples collected between Saturday and Sunday are shown in black. Sample marked in red was considered an outlier as flow measurements on that day were inconsistent. Taken from Been et al. (195).

As can be seen, benzoylecgonine loads varied greatly across the sampling campaign, in particular samples collected on the weekends. No apparent trend was visible in the data, except for five measurements during summer (July, August and September), which were lower compared to the rest of the data. However, only the three weekend samples were statistically different from the other weekend samples (Mann-Whitney test; $p\text{-value of } 0.046 < \alpha = 0.05$). Nevertheless, with regard to the large variability of measured loads, it remains difficult to determine whether this is a seasonal or a random effect.

From a law enforcement perspective, one of the first questions which rises when discussing about the utility of wastewater analysis, is whether it would be possible to detect a decrease in measured loads after a seizure. This would enable law enforcement units to get intelligence about not only the efficiency of their activities, but, above all, the impact on the structure of the drug market and on its dynamic (elasticity, inertia, etc.). However, due to the large variability observed here, it would be difficult to assess if a seizure, no matter its size, would have an impact on consumption. Furthermore, because cocaine is nonetheless a high-prevalence drug, with a substantial number of consumers (recreational and regular), one can assume the distribution network to be flexible enough to rapidly fill the shortage and meet the demand (e.g., replacement of drug dealers, opening new supply routes). It would thus be even more difficult to detect any change in consumption based on wastewater analysis.

Using the approach detailed in *Chapter V* and *Annexe III* (i.e., Monte Carlo simulations), this dataset can be used to roughly estimate the amounts of cocaine consumed daily and yearly in the city of Lausanne, as reported in Table 13.

Table 13: Back-calculated daily (pure) cocaine consumption estimated from benzoylecgonine loads shown in Figure 21.

Estimated daily cocaine consumption [g/day]

<i>Tuesday – Wednesday (n = 21)</i>	Mean	242.1
	Standard Error	47.5
	Minimum	163.4
	Maximum	410.3
<i>Saturday – Sunday (n = 21)</i>	Mean	348.2
	Standard Error	71.8
	Minimum	182.0
	Maximum	616.1
<i>All data (n = 42)</i>	Mean	301.2
	Standard Error	60.9
	Minimum	163.4
	Maximum	616.1

The estimated cocaine consumption was higher during the weekend compared to week-days. With respect to the structure of consumption, one could assume that the difference between week and weekend consumption (approximately 30% or, in absolute figures, 100 grams of pure cocaine) is due to regular (heavy) and recreational (occasional) users, respectively. Combined to data about the typology of users, this information could potentially be used to have an idea of the number of users in each group, analogously to what was done in *Chapter VI*. Yet, knowing that cocaine consumption is higher during weekends is of little use from a drug enforcement perspective, as its focus is more towards the structure of the market. If it is admitted that the estimates are representative of the consumption during week-days and weekend-days, a rough estimate of the yearly consumption could be derived. Taking for the former, the average measured between Tuesdays and Wednesdays, the yearly consumption on week-days would correspond to 63.2 kg of pure cocaine (range: 50.8-75.6 kg/year). Taking the average measured from Saturdays to Sundays as reference for weekend-days (regarded here as Saturdays and Sundays), the average yearly consumption would be 36.2 kg of pure cocaine (range: 28.7-43.7 kg/year). Summed up, the total consumption would be, on average, 99.4 kg/year of pure cocaine (range: 79.5-119.3 kg/year).

While these figures rest upon the assumption that measurements are representative of all days of the week, which has clearly not been proved, they provide a rough estimate of the size of the market in the investigated area, which would not be available otherwise. Furthermore, they can be used to define the context in which the activity of drug enforcement can be evaluated and, in particular, what seizures represent with respect to the global cocaine market. Knowing, for instance, that a specific network of drug traffickers supplies 10% of the total market is extremely useful to identify priorities and reallocate resources. Similarly, money turnover and profits generated from drug trafficking can be better evaluated. However, as already discussed on several occasions, there is a need to setup routine sampling campaigns

which will allow to continuously monitor drug use and, indirectly, the market and the criminal organisation involved.

7.2 Heroin

In *Chapter IV*, daily estimates of heroin consumption in the metropolitan area of Lausanne were presented. As a reminder, it was estimated that on average, approximately 12.7 grams of pure heroin are consumed daily. Figure 22 illustrates the amounts of heroin seized by law enforcement within the investigated catchment between the end of 2013 and 2014. As can be seen, the large majority of seizures are below 20 grams and only two pass the 100 grams threshold. In total, 4.9 kg of street heroin were seized, half of which due to a single seizure of 2.5 kg, corresponding to approximately 1.5kg of pure substance. Using as reference the estimate of daily consumption obtained from triangulation of wastewater and epidemiological data (i.e., 12.7 g/day of pure heroin), the yearly consumption would correspond to approximately 5.8 kg of pure heroin.

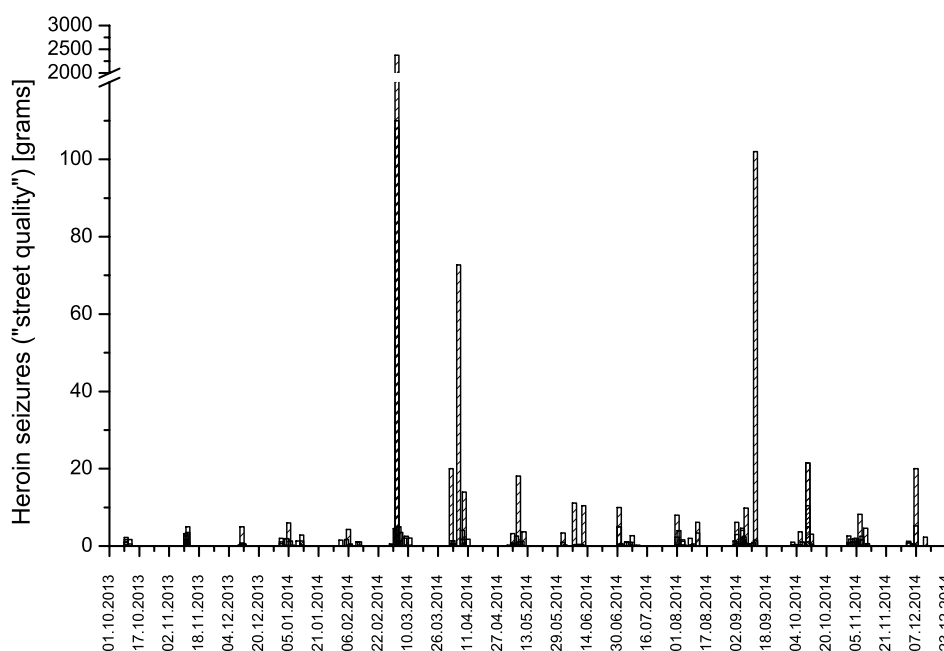


Figure 22: Chart of heroin seizures made by law enforcement in the metropolitan area of Lausanne between end 2013 and 2014.

One could think that these results have no added value for drug enforcement, except exacerbate the fact that drug enforcement seize only a fraction of what is being consumed. Yet, much more can be done with these results. To illustrate this, considered two major investigations which took place in the city of Lausanne during and after the sampling campaign considered here. The first investigation took place between between November 2013 and April 2014 and the second between December 2014 and April 2015. In both cases, the mobile phone of a drug dealer was wiretapped and, after arrest, police attempted to retroactively estimate the amounts of heroin each dealer sold during the investigation period based on phone records. As part of another research project conducted at the University of Lausanne,

a software was created, which, based on a set of criteria (e.g., when the phone call was made and how long it lasted), automatically screened phone records to determine which communications were potentially a transaction (196). Furthermore, through testimony of apprehended consumers, it was determined that heroin was generally sold as 5 g units, corresponding to 0.55 g of pure heroin (considering street heroin purity of 11% (197)). Using these figures, together with the number of detected transactions, the amount sold by each suspect could be estimated. Results are shown in Table 14. As can be seen, both were suspected of having sold, on average, approximately 6 g of pure heroin per day, which corresponds to half of the daily consumption estimated from wastewater and epidemiological data. This would therefore suggest that the heroin supply is guaranteed by only a few number of people, which is in agreement with the vision police have about the heroin market in the area. In fact, according to intelligence, the market is held by a specific network of criminals, mainly of Albanian origin, having a limited number of dealers. Combined, these information corroborate drug enforcement’s hypotheses about the structure of the heroin market in the area and justifies the scrutiny to investigate the two suspects. Concretely, this example shows that when information available to law enforcement, other than just amounts seized, are combined to wastewater and epidemiological data, it can help better understand the structure of the drug market. This definitely constitutes a valuable piece of intelligence that can feed decisions at tactical and/or operational levels. Furthermore, it also provides an alternative perspective to which results from wastewater analysis can be confronted to.

Table 14: Estimated amount of heroin sold by each suspect in the two cases investigated by Lausanne’s drug enforcement. Reproduced from (196)

<i>Case (suspect)</i>	<i>Period</i>	<i>Detected transactions</i>	<i>Estimated sales over the whole investigation (pure heroin) [grams]</i>	<i>Average sales per day (pure heroin) [grams]</i>
1	11.2013 – 03.2014	1339	536	5.1
2	12.2014 – 04.2015	991	778	6.4

7.3 Methamphetamine

Consumption of methamphetamine has recently become a topical subject in the media and among addiction researcher in Switzerland (198). In particular, in the city of Neuchâtel where the existence of a well established methamphetamine market has been known to police forces for a long time, as also shown by crime statistics in Figure 23.

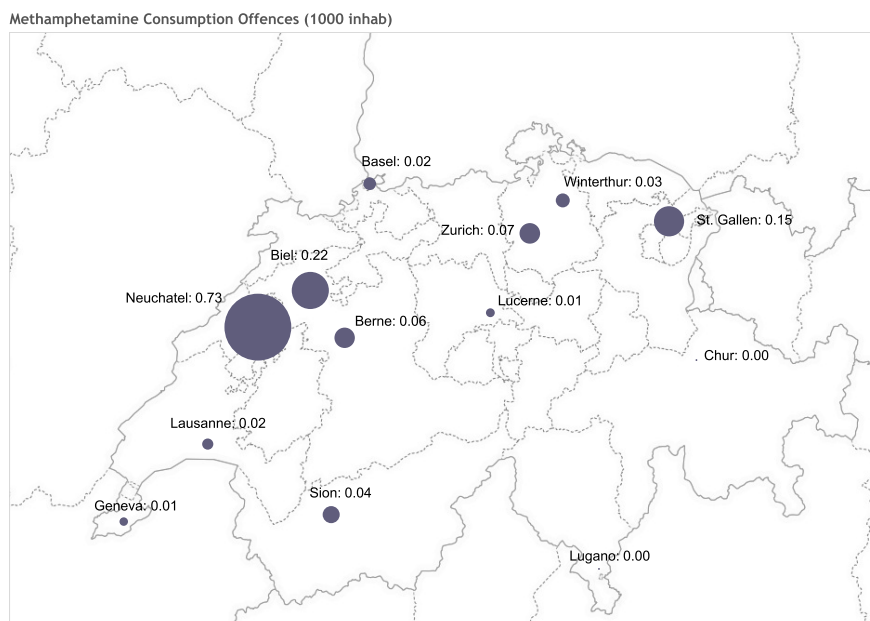


Figure 23: Reported offences for consumption of methamphetamine (normalised per thousand inhabitants) in 13 cities in Switzerland. Taken from Been et al. (185).

As already presented in *Chapter V* (see also *Annexe III*), a wastewater sampling campaign was carried out in 13 cities in Switzerland during one week in March 2014. In connection with an investigation by the police of Canton Neuchâtel, an important methamphetamine dealer was arrested in September 2014. According to the police, the suspect was accused of importing and reselling, between 2012 and 2014, approximately 8 kg of methamphetamine (i.e., “crystal”) and 4800 pills (199). Following his arrest, a request was made by the police to collect a second set of wastewater samples to determine if any change was visible in methamphetamine loads. Samples were thus collected during one week in December 2014 (approximately 12 weeks after apprehending the suspect), according to the same sampling strategy used for week in March described in *Chapter V* and *Annexe III*. Results of the first and second sampling campaign are shown in Figure 24.

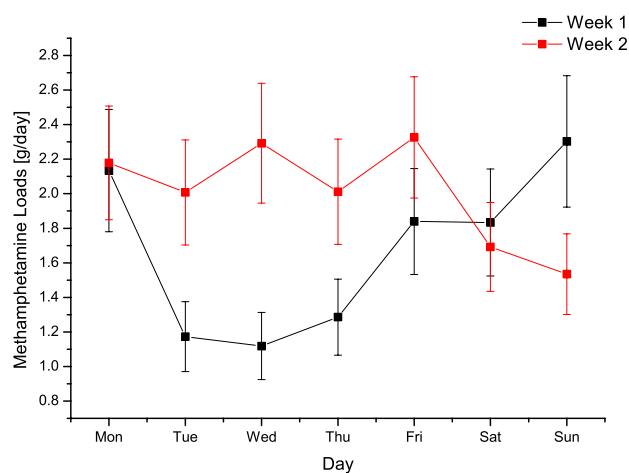


Figure 24: Methamphetamine loads measured before (Week 1, March 2014) and after arresting the suspected dealer (Week 2, December 2014).

As can be seen, no clear difference was observed in methamphetamine loads between the two sampling weeks. Average loads measured in December were slightly higher, yet no statistical difference could be highlighted (t -Test p -value = 0.08 > α = 0.05). Similarly, other drug residues (i.e., benzoylecgonine, amphetamine, MDMA and THC-COOH) did not show any difference between the two sampling weeks.

These results suggest that arresting an important supplier in the area did not have a major effect on methamphetamine consumption, at least according to wastewater analysis. This result could be perceived as an indication that the work of law enforcement has no impact or that wastewater analysis is not able to reflect such a change. However, this could also mean that the market had sufficient stocks and/or other supply routes existed or were established flowing the arrest of the suspect. It should be kept in mind that the second set of analyses were performed twelve weeks later, during which time the methamphetamine shortage could have been filled. This is particularly interesting when the results are placed in a broader perspective. In Figure 25, the results from the one week sampling campaign conducted in March 2014 in 13 cities in Switzerland are reported (see *Chapter V* for further details). It can be seen that in the nearby cities of Biel and Bern, noteworthy methamphetamine per capita loads were measured compared to the Swiss average. In this respect, the hypothesis formulated previously, that consumers and/or dealers had access to other supply routes, becomes even more plausible. These findings are in agreement with the hypothesis, based on police intelligence, that in the area around Neuchâtel, Biel and Berne, there is a well established methamphetamine market.

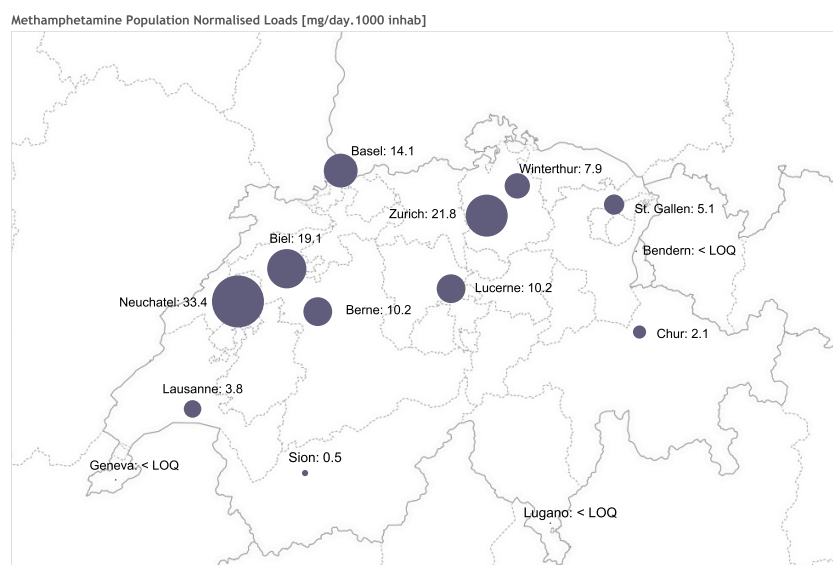


Figure 25: Average population normalised methamphetamine loads [mg/day.1000 inhabitants] measured in March 2014 in 13 cities in Switzerland and in Liechtenstein. Taken from Been et al. (185).

7.4 Major findings and conclusions

Through the three examples presented here, it has been shown that wastewater analysis clearly has an added value also from the perspective of law enforcement. It is however important to identify how the findings can be employed and not simply stop at the observation that drug enforcement units seize only a fraction of what is being consumed or that their activities have little impact on the market. When wastewater is combined with appropriate data, it can help drug enforcement units to evaluate the extent of the drug market, the size of the user population, and corroborate (or confute) their current beliefs about how trafficking networks are structured. Furthermore, within the limits of the considered situations, these results show that, although influenced by their own strategies, drug enforcement have a relatively good view of the situation. Finally, information arising from police investigations allows also to strengthen results derived from wastewater analysis, which as it has been pointed out on several occasion, are also affected by various uncertainties.

VIII Sibutramine – An example of application in the field of public health

Sibutramine (1-[1-(4-Chlorophenyl)cyclobutyl]-N,N,3-trimethyl-1-butanamine) is an anorexiant which was withdrawn from the European market because of its adverse effects (200). However, it is still being used in illegal or counterfeit pharmaceuticals (201) and, according to the Swiss Agency for Therapeutic Drugs (Swissmedic), parcels containing the substance in various forms are being regularly seized at borders in Switzerland (Swissmedic, personal communication). However, very little is known about the prevalence of use of these products in the general population.

This part of the research consisted in a preliminary study aimed at determining if consumption of sibutramine could be assessed through the analysis of wastewater samples. Focus was set on sibutramine and two of its urinary metabolites, monodesmethylsibutramine and didesmethylsibutramine (202). For this purpose, specific extraction (SPE) and analytical methods had to be developed. Wastewater samples were collected at the WWTP of Lausanne between August and October 2014, according to the same sampling scheme detailed in *Chapters IV and VI* (24 hour composite samples from 12 p.m. to 12 p.m.).

8.1 Sample preparation and instrumental analysis

The methodology used to process wastewater samples was inspired by existing procedures used for the analysis of sibutramine and its metabolites in urine (203–205). One-hundred millilitres of 24 hour composite sample were spiked with stable isotope-labelled internal standards of the target compounds, filtered (0.7 μm nitrocellulose filters, type GF/F Whatman, GE Healthcare), acidified to pH 3 using hydrochloric acid and extracted using Oasis HLB SPE cartridges (3cc, 60mg, Waters, Milford, MA, US). Elution was carried out using 4mL of methanol. One millilitre of the eluate was then evaporated to dryness at room temperature under a gentle flow of nitrogen and reconstituted in 1mL of water (with 0.1% of formic acid) and methanol (90:10 volume/volume). Analyses were performed on an Agilent 1290 UHPLC system (Agilent, Santa Clara, CA, US) coupled to 5500 QTrap triple quadrupole mass spectrometer (ABSciex, Ontario, Canada) interfaced with an electrospray ionization (ESI) operated in multiple-reaction-monitoring (MRM) mode, in positive ionisation. The parameters of the MS/MS method are reported in Table 15. Separation was carried out using a Kinetex Core-Shell C18 column (particle size 2.6 μm , 100 x 2.1 mm, Phenomenex, Torrance, CA, USA) at a constant flow rate of 0.8 mL/min.

Table 15: Summary of main parameters of the instrumental method. Rt: Retention time, IS: Internal standard.

<i>Compound</i>	<i>Rt</i> <i>[min]</i>	<i>Parent</i> <i>[m/z]</i>	<i>Fragment</i> <i>[m/z]</i>	<i>Collision</i> <i>Energy</i> <i>[V]</i>	<i>IS</i>
Sibutramine	2.98	280.1	125.0	36.0	Sibutramine-d7
			139.0	22.0	
Desmethylsibutramine	2.85	266.1	125.0	31.0	Desmethylsibutramine-d7
			139.0	19.0	
Didesmethylsibutramine	2.74	252.2	125.0	27.0	Didesmethylsibutramine-d7
			138.9	15.0	

Linearity, accuracy and intermediary precision were determined on three consecutive days using blank tap water samples spiked at three different concentrations (i.e., 3, 10 and 100 ng/L) (206). Calibration was performed between 2 and 200 ng/L. Extraction recoveries were determined by spiking wastewater samples (n=3) with deuterated standards before and after solid-phase extraction (207,208). Results of method validation are reported in Table 16. Method quantitation limit was set as the concentration of the lowest calibration level. Because this was only a preliminary study, method detection limit was not assessed extensively. Still, the signal-to-noise ratio measured at 2 ng/L concentration was high (> 500) for all target analytes, suggesting that the actual limit of detection is substantially lower.

Table 16: Summary of validation results. Concentration: concentration of the validation samples; MDL; method detection limit; MQL: method quantitation limit

<i>Compound</i>	<i>Concentration</i> <i>[ng/L]</i>	<i>Accuracy</i>	<i>Intermediary</i> <i>Precision</i>	<i>R</i> ²	<i>% Recovery</i> <i>(SD)</i>	<i>MDL</i>	<i>MQL</i>
Sibutramine	3	3.4%	4.8%	0.9996	70.1 (20.5)	< 2 ng/L	2 ng/L
	10	4.1%	2.8%				
	100	1.8%	3.8%				
Desmethylsibutramine	3	3.3%	6.0%	0.9999	76.3 (28.9)	< 2 ng/L	2 ng/L
	10	3.8%	5.3%				
	100	0.8%	3.8%				
Didesmethylsibutramine	3	2.1%	5.2%	0.9992	82.4 (15.7)	< 2 ng/L	2 ng/L
	10	4.0%	5.3%				
	100	1.4%	2.8%				

8.2 Analysis of sibutramine in wastewater samples

As described previously, seven 24 hour composite samples were collected between August and October 2014. The target analytes could not be detected in any of the samples. Very little is known about the excretion rate of the target compounds after consumption of sibutramine. Further studies should be carried to determine to which extent these residues are found in urine and if they are excreted as phase II metabolites (i.e., glucuronides), which were not targeted in this study. Moreover, no stability experiments were carried out in this context.

Nonetheless, the negative results seem to indicate that consumption of prohibited pharmaceuticals containing sibutramine is likely limited in the investigated area. Another aspect which supports this claim is that recipients of the seized parcels were generally located in the north-eastern part of Switzerland, whilst samples analysed here were collected in western part of the country. This could however also be interpreted as an indication that Switzerland is only a transit country for this type of products.

8.3 Major findings and conclusions

The results from this preliminary study show that sibutramine and its urinary metabolites were not detectable in samples collected at the WWTP of Lausanne. Although some unknowns remain, regarding the excretion of these products and their stability in wastewater, these first results suggest that, at least in the investigated area, consumption of these illegally imported pharmaceuticals is likely limited. This is an interesting example which shows the potential application of WBE to determine if harmful, illegal and/or counterfeit pharmaceuticals are being consumed by the general population. Combined to surveillance of online pharmacies and information from seizures, targeted analysis could be carried out to detect other dangerous substances.

IX Discussion

9.1 Long-term monitoring of illicit drug consumption

The possibility to monitor illicit drug use over time and detect potential changes is likely the most powerful application of WBE. However, a clarification has to be made as to what is meant by “detecting changes”. In this context, these should be understood as substantial and enduring modifications in drug use, detected through the analysis of wastewater samples collected over longer periods of time (e.g., months and years). These should be distinguished from short-term or punctual changes, due for instance to specific events such as seizures, which will remain difficult to detect with WBE. In fact, unless changes in loads are substantial and lasting, it will be difficult to differentiate them from the “natural” day-by-day variability observed in drug consumption. Furthermore, in large catchments (i.e., cities with tens of thousands inhabitants or more) and for high-prevalence drugs (e.g., cocaine and cannabis), distribution networks are likely large and flexible enough to rapidly fill potential supply shortages, making these events imperceptible through wastewater analysis. For smaller catchments or less prevalent drugs (thus having smaller distribution networks), punctual events might be easier to detect. Yet, for particularly small catchments (i.e., villages), the limited number of drug users and/or the short residence time, which in terms limits analyte dispersion in sewers, further increase the day-by-day variability of drug loads (209).

Whilst, for the above reasons, detecting punctual events remains difficult, WBE could prove useful to detect and monitor durable changes in drug use and, from a prevention and repression perspective, evaluate the long lasting effect of measures and interventions. Contrarily to surveys, which take time to perform and thus only provide retrospective information, data from WBE could be retrieved rapidly and would provide an objective indication of what is happening. An example of this was shown in *Chapter VI*, where wastewater analysis provided evidence that recent changes in cannabis regulations did not seem to affect consumption. A further example is given by results from monitoring campaigns in Australia, where since 2011, researchers have detected an increase in methamphetamine consumption (210). Wastewater-based epidemiology could also prove useful as an additional early-warning system to detect that a specific substance is being increasingly replaced by another, more dangerous one. Or, it could serve to detect the use of a new and previously undetected substance, provided the compound is being monitored or that the analytical method is sensitive enough to detect it (see *Section 2.4.4*). These instances would likely be less rapidly detected with other indicators such as surveys, police or intoxication statistics. Furthermore, specific harm-reduction or preventive measures can be planned in response to observed changes detected through wastewater analysis. However, these examples do not diminish the importance of conventional methods, which provide extremely useful information that cannot be gathered by WBE, especially when trying to understand reasons, structure and potential consequences of the observed changes.

Carrying out long-term sampling campaigns in the same catchment has some additional advantages. In particular, sewer architecture, but also in-sewer transformations of biomarkers, can be considered constant over time and thus do not need to be accounted for when analysing potential changes. Furthermore, fluctuations in the size of the population have also been shown to have a limited influence on drug loads, mainly because the variation of the latter are orders of magnitude higher compared to population markers (76,144). The only exception being public events, which will attract a large number of people from outside the catchment over a brief period of time. Nevertheless, in a long-term monitoring perspective, this kind of event have only a limited relevancy. Furthermore, the occurrence of such events will generally be known and they can be accounted for in the interpretation of the results. A final and important aspect which has to be considered when planning long-term campaigns is the sampling design. The latter should be adapted to the question one seeks to answer, the target substances, the size of the catchment and the degree of uncertainty deemed acceptable (209). Overall however, collecting samples over the whole period of interest according to a stratified random design has been shown to be a more adequate approach compared to consecutive sampling (e.g., a couple of weeks per year), if the goal is to detect trends or compute yearly estimates (209).

As will be discussed in the following section, these factors will play an important role when investigating patterns of drug use at different locations.

9.2 Assessment of geographical differences in illicit drug use

Understanding the geographical differences in illicit drug use is particularly important from a global perspective. Identifying which drugs are being used and where their use is more widespread, can help to better understand how the market and its supply routes are structured. From a prevention perspective, it can help to identify future consumption habits (e.g., shift towards a more convenient drug imported from a nearby country/region) and to adapt strategies accordingly, and this at regional, national and international level. From the repression viewpoint, this kind of information can be used to better understand how criminal organisations operate, which are their supply routes and target markets. The United Nations Office for Drugs and Crime (UNODC) and the International Narcotics Control Board (INCB), together with the EMCDDA in Europe, are among the leading actors in the global monitoring of drug markets. Their vision is mainly based on indicators described in *Section 1.1* (e.g., surveys and seizures), yet combining data from different countries, often having their own reporting criteria, is not an easy task. In this maze of indicators, wastewater analysis could prove to be a useful indicator to combine with the more traditional ones and overcome some of these issues. Yet, comparative studies based on WBE also have their limitations. Whilst the occurrence of drug residues in sewers is per se an objective measure of drug use, its measurement is affected by various sources of uncertainty, making direct comparisons more difficult. From an analytical perspective, best practices and external quality controls have been setup to guarantee that laboratories achieve comparable results when participating in international studies (i.e., Sewage Analysis CORE Group Europe (SCORE)) (31,146). However, the major issues are related to aspects which the analyst cannot control, and

probably never will. Among these are the different sampling procedures and flow measurement techniques used by WWTP, as well as the architecture of the investigated sewer system. Specific questionnaires have been developed to gain as much information as possible (146), but accounting for all these factors still remains a difficult task. Different sewers could also have different effects on the stability of target analytes (119). One way to, at least partially, overcome some of these issues is to collect more samples, as well as measuring population markers. For the moment, however, it is the analyst, with the help of various statistical tools, who is called to assess to which extent differences in measured loads can be linked to differences in use. However, when these differences are substantial and, thanks to the information gathered through the questionnaires, sampling procedures and sewer architecture are known not to differ significantly, one can reasonably infer that the observations are likely due to differences in the structure of consumption (i.e., number of users, consumption per user or substance purity). Most importantly, however, if such differences are identified, more specific investigations can be carried out (e.g., cohort studies, questioning users or field workers) to determine their origin and, if deemed necessary, plan interventions (e.g., prevention and harm-reduction campaigns or repressive measures).

9.3 Triangulation with other sources of data and back-calculations

The major distinction which can be made between conventional methods used to monitor illicit drug use (e.g., surveys, police statistics, drug related deaths, treatment demands) and wastewater analysis is that the former provide directly, or indirectly, information about *consumers*, while the latter is the only one providing direct information about *consumption*. Nonetheless, the complexity of drug use is such that any indicator will always provide only “a partial, yet informative, perspective of the phenomenon” (p. 1, (185)). Inevitably, this results in the need to triangulate the various sources of data, to benefit of their complementarity and attempt to better understand this hidden and complex phenomenon.

Throughout this research, data triangulation has been shown to be a crucial way to overcome some of the individual limitations of the available indicators about drug use. Issues related to surveys (e.g., concealment, lack of data about heavy drug users, recall bias), which, in summary, tend to underestimate prevalence and thus consumption, have been highlighted in several respects. Whereas these methods will never become perfect, they will always be among the most valuable tools for addiction researchers to assess the current situation and plan future strategies. The gathered information is also highly relevant to the wastewater-based epidemiologist, who confronted to data affected by various (partly still unknown) sources of uncertainty, can use it to help her interpretation.

Although great improvements have been made in understanding the mechanisms which affect occurrence and stability of drug residues in sewers, some aspects, and their mutual effects, will likely never be fully understood, or at least, not in the nearest future. As for any measurement, uncertainty will always be part of WBE, yet its integration in the panoply of existing indicators will certainly help shed light on some of these unknowns. These

considerations are also relevant for back-calculations, which, although being subject to additional uncertainties, represent the perfect form in which wastewater data can be combined to epidemiological figures to make inferences about drug *consumption* and *consumers*. Only through comparison with other data sources, one can evaluate if the obtained estimates are plausible or not. However, the purpose of data triangulation goes beyond corroborating (or confuting) the validity of the obtained figures. In fact, if several indicators all point towards the same direction, like the case of heroin presented here (see *Chapters IV* and *VII*), this presupposes that the population of users can be measured and, most importantly, is known. This piece of information is highly relevant for institutions and social workers as it suggests that the dark figure of marginalised users that are not captured by existing prevention and harm-reduction measures is limited. On the contrary, for substances whose consumption has various facets (i.e., infrequent, recreational versus regular and heavy users), such as cocaine and cannabis, triangulation of wastewater data with other indicators could potentially allow to estimate the dark figure of heavy and problem users which evade treatment, prevention and harm-reduction programs. In fact, the model presented in *Chapter VI* for cannabis, could serve to assess the proportion of heavy users, which might develop a problem drug use in future. Similarly, if information about average consumption of user types (available from surveys or assessed from expert opinion) would be combined to estimates of total consumption (determined via WBE and/or other indicators), one could construct various scenarios to evaluate the proportion of users which would most likely fall in each category.

However, research is still necessary to understand which and how multiple data sources should be integrated in a global model allowing to gauge drug use at the community level. “Translating” epidemiological and wastewater data in such a way that these can be integrated in a single framework is far from being straightforward. Widely used in (drug) epidemiology, Bayesian inference models are likely the method of choice to combine data sources and quantify their uncertainty in a formal approach.

For all of the above reasons, wastewater-based epidemiologists should always closely collaborate with epidemiologists, addiction researchers, pharmacologists, toxicologists, forensic scientists, but also law enforcement, to interpret their data in a “truly transdisciplinary approach” (211).

9.4 From a drug enforcement viewpoint

Whilst there is no doubt that the vision of law enforcement is strongly influenced by their strategies and focuses, themselves influenced by external pressure such as public opinion and politics, their work offers an interesting vision on both drug demand and supply (194). This was demonstrated in *Chapter VII*, where estimates about heroin consumption derived from WBE and epidemiological data suggested that heroin consumption and trafficking were limited in the area. Intelligence from drug enforcement, derived from investigations and informants (mainly consumers), seemed to corroborate this hypothesis. Moreover, it was shown that WBE can be used to derive highly relevant intelligence which allowed to better understand the methamphetamine market in a specific area. In fact, knowing that its consumption remained steady, or that supply shortages were rapidly filled, after arresting one of the major dealers is

highly relevant for drug enforcement. Namely, these findings suggest that this particular market consists of a well established network of regular and heavy users with access to various supply routes.

Thus, from a drug enforcement perspective, the findings presented in *Chapter VII* show that WBE can be potentially useful to i) assess the size of the market, ii) define the context in which the effect of police actions can be evaluated, iii) quantify the revenues of drug trafficking, iv) provide clues about how criminal networks operate and iv) guide decision-making processes such as defining strategies and relocating resources. Vice versa, police's image about the drug market can help both conventional and wastewater-based epidemiologists evaluate their findings from a different perspective. This can be extended to public health problems related to the use of NPS. In fact, law enforcement and, in particular, customs, are among the few having information about the use of these substances. Because of the limited number of pharmacokinetic studies available and the ever growing number of molecules being synthesised, screening for these substances in wastewater remains challenging. Surveys also face various limitations, mainly because users often do not have full knowledge of what they have consumed. Seizures could be used as guideline to identify substances, whose consumption in the general population should be further investigated. Targeted wastewater analysis could then be used, eventually allowing to determine if consumption in the general population is of concern or not.

9.5 Perspectives and future applications

9.5.1 Markers of substance use and lifestyle

The focus of the present research was set on conventional illicit drugs, in particular cocaine, opioids, cannabis and amphetamine-type stimulants. The potential of applying WBE to the field of new psychoactive substances has already been discussed. However, illicit drugs are not the only group of xenobiotics, whose (mis)use at the community level could be monitored via WBE.

Analysis of pharmaceuticals with potential for abuse (e.g., opiates, benzodiazepines), a field closely related to illicit drugs, has also been investigated (120). However, WBE could also be employed to determine the extent of illegal and counterfeit pharmaceutical use. Examples of applications are the analysis of sibutramine (see *Chapter VII*) as well as the study by Venhuis et al. (212). In their work, the authors determined that sildenafil consumption, based on wastewater measurements, was substantially higher than reported in official sale figures. This suggested that a large portion of pharmaceuticals used are purchased through unofficial (illegal) channels (e.g., online pharmacies). Similarly, the monitoring use of "smart drugs", i.e. substances used to enhance intellectual performances, through wastewater analysis has also been investigated (213,214). These few, although promising, examples show that WBE could be successfully implemented to measure the extent of drug diversion. The utility of this approach becomes even clearer if one considers the increasing number of online pharmacies, freely selling prescription or, worse, counterfeit products (215).

Doping agents, or more generally performance enhancing drugs, are closely related to online pharmacies, as these products are also often bought online. Monitoring the use of dopants among professional athletes is extremely complex. The numerous methods used and the advent of micro-dosing of recombinant human erythropoietin, make it difficult to reveal prohibited practices, even with the introduction of the “biological passport” (216,217). However, doping does not seem to be a phenomenon limited to professionals, as it also touches amateur athletes (218). In this context, WBE could provide clues about the extent of use of these substances in the general population, in particular for prohibited products such as anabolic steroids. To date, a single study investigating the occurrence of these substances in wastewater has been published (219). The major issue however, is that many of these substances are either legally available (e.g., ibuprofen) or naturally excreted (e.g., hormones). Furthermore, some of the illegal substances used as dopants, are also used in a recreational context (e.g., cocaine, amphetamine, cannabis (218)), making the interpretation of the results extremely complex. For this reason, applications of WBE to monitor doping in the general population will likely be limited to illegal and exogenous products (e.g., exogenous steroids).

9.5.2 Markers of health and exposure

Monitoring of public health in a broader sense, has been identified by researchers as a highly promising field where WBE could be successfully implemented (220). In recent years, the World Health Organisation reported increasing prevalence and incidence of deaths related to chronic and high-prevalence diseases such as diabetes as well as lung, colorectal and breast cancer (221,222). These can also be caused by the exposure to external contaminants like pesticides, plasticisers and UV-filters, just to mention a few, all of which have been shown to have potentially adverse effects (e.g., endocrine disruptors, carcinogenics). From a public health perspective, monitoring the evolution of diseases in the general population will become more and more important, as some of these will affect an increasing number of individuals due to lifestyle, diet and exposure to contaminants. The recent improvements in the capabilities of advanced chromatographic and mass spectrometric techniques have opened the way to the discovery of an ever-growing number of clinically relevant molecules in urine. However, the large number of undiagnosed cases and the high costs of setting up large urine screening campaigns make it a difficult task, in particular when it comes to assessing the prevalence among the general population which has not yet developed any noticeable symptoms. Wastewater-based epidemiology provides the opportunity to develop sophisticated and innovative monitoring strategies to gather almost real-time information about public health directly at the population level. In comparative studies, it could be used to assess or confirm that differences in disease levels exist between communities exposed to distinct environmental conditions. Furthermore, it could be used as early-warning system to detect the first signs of upcoming diseases, thus delivering relevant information to health officials who would be able to plan countermeasures. Similarly, it could provide useful information to assess the effect of new strategies adopted to tackle the spreading of these conditions. Currently, only few applications have been reported in the literature. One focused on the development of DNA biosensor for online measurements in sewers, which could potentially be used as an

indicator of disease levels in communities (223). Another study reported the development of a large-volume injection LC-MS/MS method for the analysis of nucleosides in wastewater (224). Two additional studies have recently been published which focused on the analysis of F2-prostaglandins in wastewater using enzyme-linked immunosorbent assays (ELISA) (225) and LC-HRMS (226).

X Conclusion

The possibility of analysing wastewater to access information about the consumption of illicit drugs at the community level is a very young approach, which has however experienced an astonishingly rapid growth in the past decade. During this period, numerous groups around the world started researching in the area and quickly recognised its potential. Initial efforts were clearly focused on developing sophisticated analytical techniques, required to precisely quantify trace amounts of drug residues in an extremely complex matrix. The wide resonance of the first results sparked the interest of epidemiologists and addiction researchers, who rapidly joined ranks and helped acknowledging this innovative approach. A network of institutions and laboratories, striving to implement and further improve the method, was then created. Since then, research has broadened and topics such as in-sewer transformations, identification of new metabolites, data triangulation and the analysis of health and exposure biomarkers are now being extensively explored. Whilst remaining a strong transdisciplinary approach, the achievements and the widespread recognition have helped elevating wastewater-based epidemiology to the rank of discipline, whose potential is far from being fully exploited.

The objective of this research was dual. The first, implicit, goal was to initiate a research path on wastewater-based epidemiology at the *Ecole des Sciences Criminelles* of the University of Lausanne. This consisted in gathering the necessary knowledge about the techniques to be able to collect, process and analyse wastewater samples and, above all, to work on ways to produce intelligence from the analytical results. The main, explicit, goal of this research was to evaluate the added value of wastewater-based epidemiology to measure illicit drug use. The methodology chosen was based on the assessment of the informative value extracted from wastewater samples, and triangulating results from various sampling campaigns with existing indicators about drug use. Although affected by various sources of bias, conventional indicators have been used and refined for decades, thus representing the ideal landmark to evaluate the added value of wastewater-based epidemiology.

The focus of this research was the metropolitan area of Lausanne, in western Switzerland, and its population. Using the best available knowledge about wastewater sampling and analysis, a procedure was setup to collect and analyse samples from the city's wastewater treatment plant. The objective being the triangulation with existing data, it was decided to focus on "conventional" drugs such as cocaine, heroin, amphetamine, methamphetamine, MDMA and cannabis. The initial phase of the work centred on evaluating short- and long-term fluctuation of selected drug residues in wastewater and determining if and how the population, monitored through the measurement of ammonium, influenced drug load patterns. By the analysis of this population marker, population dynamics could be highlighted (e.g., commuters), but simultaneously it could be determined that these variations are negligible compared to the "natural" variability of drug loads. Findings thus suggest that, at the temporal

scale considered here (i.e., months to a few years), variability in drug use is orders of magnitude larger compared to population dynamics. The only exception being major public events, where both drug use and number of contributing people can substantially deviate from “normality”.

Subsequently, this work focused on trying to validate the selected sampling design using a specific substance, methadone, for which allegedly precise prescription data was available. Using Monte Carlo simulations to assess the uncertainty of the estimates, it was possible to show that wastewater-based figures were in line with expectations from prescription data. These results validated the selected sampling strategy as being adequate to reliably monitor the consumption of a relatively small group of individuals. The analysis was then extended to heroin, for which epidemiological data was available. On the one hand, it was shown that current knowledge about the excretion of 6-monoacetylmorphine, heroin’s specific metabolite, did not allow to obtain realistic estimates of consumption. On the other hand, morphine loads combined to prescription and sales data provided estimates of heroin consumption in agreement with figures derived from epidemiological data. This was shown to be useful not only to measure quantities of heroin used, but also to acquire additional evidence about heroin prevalence. Furthermore, it highlighted that current harm reduction programs efficiently target injecting drug users.

The successive step was carried out in collaboration with various institutions in Switzerland and abroad. The result was an extensive study of geographical differences in drug use based on wastewater, survey and crime data in Germany and Switzerland. Taken individually, surveys and crime statistics depicted substantially different images of cocaine and heroin use. Concealment, stigmatisation, visibility of the drug scene, public opinion and law enforcement strategies were called into question to explain the discrepancies. Wastewater analysis proved to be very useful to untangle this complex picture. It was shown that, whilst law enforcement strategies and visibility of the drug scene likely differed between locations, consumption of cocaine and heroin was quite homogeneous across Switzerland. For amphetamine-type stimulants, a good overlap was observed between all data sources. Wastewater confirmed the existence of some hotspots of methamphetamine use, but also identified a more widespread use of amphetamine in northern and eastern parts of the country.

By focusing on the most consumed illicit drug, cannabis, the fourth part of this work centred on further understanding how wastewater data could be integrated with existing statistics to refine current knowledge about prevalence of use. At present, this is a highly topical subject due to the ongoing scrutiny of current legislations. However, monitoring cannabis use via wastewater is a difficult task because of analytical issues and the scarce literature about the drug’s pharmacokinetics. By integrating various sources of data, and accounting for their respective uncertainties, it was possible to obtain an updated and refined estimate of prevalence of cannabis use in the metropolitan area of Lausanne. While still being affected by various sources of uncertainty, these figures provide a quantitative indication of what the actual prevalence of cannabis use might be in an urbanised area in Switzerland.

Confronting the obtained results with law enforcement was a crucial element of this research, and resulted in a rewarding exchange of information. Combined to other research conducted at the *Ecole des Sciences Criminelles*, it was shown that wastewater analysis is an additional tool that helps understanding the structure, size and revenues of drug trafficking networks. Furthermore, the potential to apply the approach to other fields, such as monitoring the use of prohibited substances, has also been proved. The privileged position of *Ecole des Sciences Criminelles*, at the crossroads of criminology and forensic sciences, academic research and operational activities, constituted the ideal ground for the accomplishment of this research.

The results obtained in the present work have shown that wastewater-based epidemiology is a powerful approach with numerous scopes. Measuring a complex phenomenon like illicit drug use remains a challenging task and wastewater-based epidemiology certainly is not the solution to all issues. Further research is needed to improve current understanding about occurrence of drug residues in sewers, as well as extend its application to other fields. However, it has already proved itself an invaluable tool and, without the pretention of substituting existing indicators, should as such be placed among the recognised methods used to monitor illicit drug use at the regional, national and international scale.

XI Annexe I: Population normalization with ammonium in wastewater-based epidemiology: Application to illicit drug monitoring

Been F., Rossi L., Ort C., Rudaz S., Delémont O., Esseiva P., *Population Normalization with Ammonium in Wastewater-Based Epidemiology: Application to Illicit Drug Monitoring* Environmental Science & Technology, 2014, 48 (14), 8162–69; <http://dx.doi.org/10.1021/es5008388>

11.1 Abstract

Fluctuations in ammonium (NH_4^+), measured as $\text{NH}_4\text{-N}$ loads using an ion-selective electrode installed at the inlet of a sewage treatment plant, showed a distinctive pattern which was associated to weekly (i.e., commuters) and seasonal (i.e., holidays) fluctuations of the population. Moreover, population size estimates based on $\text{NH}_4\text{-N}$ loads were lower compared to census data. Diurnal profiles of benzoylecgonine (BE) and 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH) were shown to be strongly correlated to $\text{NH}_4\text{-N}$. Characteristic patterns, which reflect the prolonged nocturnal activity of people during the weekend, could be observed for BE, cocaine, and a major metabolite of MDMA (i.e., 4-hydroxy-3-methoxymethamphetamine). Additional 24h composite samples were collected between February and September 2013. Per-capita loads (i.e., grams per day per 1000 inhabitants) were computed using census data and $\text{NH}_4\text{-N}$ measurements. Normalization with $\text{NH}_4\text{-N}$ did not modify the overall pattern, suggesting that the magnitude of fluctuations in the size of the population is negligible compared to those of illicit drug loads. Results show that fluctuations in the size of the population over longer periods of time or during major events can be monitored using $\text{NH}_4\text{-N}$ loads: either using raw $\text{NH}_4\text{-N}$ loads or population size estimates based on $\text{NH}_4\text{-N}$ loads, if information about site specific $\text{NH}_4\text{-N}$ population equivalents is available.

11.2 Introduction

In wastewater-based epidemiological studies, in particular, for illicit drug monitoring campaigns, scientists have highlighted the importance of developing a mean to account or estimate the fluctuations in the size of the investigated population. This is important in longitudinal or comparative studies to verify whether fluctuations observed in illicit drug loads are due to changes in consumption habits (e.g., increased/decreased consumption, product purity, effect of drug policies, and prevention campaigns) or if they are the result of variations in the size of the population. Additionally, these tools are used to obtain reliable estimates of the actual size of the contributing population (i.e., de facto population). Census data (i.e., de

jure population) and/or sewage treatment plant (STP) design capacity are static and do not take into account fluctuations during sampling (e.g., commuters, holidays, and demographic changes) (122,227). Water quality parameters, such as biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP), have been used to estimate the population (53,146). These parameters are routinely monitored by STP laboratories. The major drawback is that there can be other sources, such as industrial discharges, which could bias the estimated size of the population (137,138). This could explain why very large variations in the estimated size of the population were measured (53). Pharmaceuticals, personal care products, and various human metabolites, measured in wastewater samples, have also been used to estimate the size of the population (125,138,142,143,157). Contrary to water quality parameters, these compounds are more specific because they are direct indicators for human activity and metabolism. However, they often require different sample preparation steps from those used for illicit drugs, thus introducing additional time consuming procedures, and currently cannot be measured online in sewers.

Ammonium (NH_4^+), the major form in which ammonia (NH_3) is found in water, is an indirect marker of urine (38,40), because it is mainly due to hydrolysis of urea (148), and is predominantly introduced in sewers via toilets (39). This particular compound is highly interesting for wastewater studies because it is supposed to be less affected by non-human sources compared to conventional water-quality parameters (53) (e.g., COD, BOD, TN, and TP). Additionally, in comparison to pharmaceuticals, personal care products, and human metabolites, this parameter can be measured at high resolution directly in the wastewater stream using an ion-selective electrode (ISE).

The scope of this study was to evaluate if $\text{NH}_4\text{-N}$ loads, measured directly in the wastewater stream, could be used i) to obtain estimates of the size of the population and capture its fluctuations at different time scales (i.e., weekly and monthly), ii) to determine if a correlation could be established between diurnal patterns of $\text{NH}_4\text{-N}$ and target illicit drug residues, and iii) if fluctuations occurred in the population (on the basis of online measurements of $\text{NH}_4\text{-N}$), over longer periods of time (i.e., months), to determine how these affect illicit drug loads. This would be of particular interest for long-term monitoring campaigns during which marked changes in the population could occur.

11.3 Materials and methods

11.3.1 Target compounds

In the scope of this work, focus was set on cocaine (COC) and its major metabolite benzoylecgonine (BE), the major metabolite of cannabis (11-nor-9-carboxy- Δ 9-tetrahydrocannabinol or THC-COOH), ecstasy [(±)-3,4-methylenedioxymethamphetamine or MDMA], and one of its major metabolites 4-hydroxy-3-methoxymethamphetamine (HMMA). See *Section 11.6.2* for additional information about standards and chemicals.

11.3.2 Sampling domain

Measurements and sampling were carried out at the inlet of the STP (upstream of the primary settling tanks) that serves the metropolitan area of Lausanne for a total residential population of approximately 220'000 inhabitants (P_{census}), according to the latest census data (150) (on the basis of the number of registered inhabitants for October 2013). The investigated sewer system is predominantly gravity drained on the basis of a combined system, and the average residence time of wastewater is approximately 2h (228).

11.3.3 Ammonium and flow measurements

Ammonium measurements were carried out using an ISE (AmmonoLyt Plus 700 IQ, WTW, Xylem, Inc., White Plains, NY) which measures $\text{NH}_4\text{-N}$ and K^+ ions directly in the wastewater stream. According to manufacturer specifications, the measurement range of the device is 0.1-100 $\text{mg}\cdot\text{L}^{-1}$ $\text{NH}_4\text{-N}$ (229). Concentrations were measured every minute between January and September 2013. See Figure 32 of the Supporting Information for a plot of measured concentrations. Matrix adjustment of the ISE was carried out by the STP personnel every 2 weeks. Flow data (measured at the inlet in m^3s^{-1} and averaged over 5 min) for the same period of time were provided by the STP personnel.

11.3.4 Wastewater sampling, preparation, and chemical analysis

All samples were collected using an autosampler (6712FR ISCO refrigerated autosampler, Teledyne ISCO, Lincoln, NE) equipped with 24 PP bottles (1 L) operated at 4°C. A total of 65 mL of sewage water was collected every 5 min, so that each (hourly) bottle consisted of 12 x 65 = 780 mL. Two 24h profiles, collected during dry weather conditions, were obtained by analyzing each of the collected bottles separately and multiplying the determined concentrations by the corresponding flow.

Additionally, 11 composite samples (24h) were collected between February and September 2013 to investigate the effect of normalization with $\text{NH}_4\text{-N}$ on the longitudinal evolution of illicit drug loads measured in wastewater samples. Daily 24h composite samples were obtained by manually mixing hourly composite samples in a volume-proportional manner. See Table 17 of the Supporting Information for details about the sampling campaign.

After collection, wastewater samples (500 mL) were immediately filtered (0.7 μm nitrocellulose filters, type GF/F Whatman, GE Healthcare), acidified to pH 2 using 37% hydrochloric acid, and extracted using Oasis MCX (Waters, Milford, MA) solid-phase extraction (SPE) cartridges (3 cc, 60 mg). Analyses were carried out using a gas chromatograph coupled to a triple quadrupole mass spectrometer (GC-MS/MS, 7890A gas chromatograph and 7000 triple-quadrupole mass spectrometer, Agilent, Santa Clara, CA) in multiple reaction monitoring (MRM) mode. See *Sections 11.6.3* and *11.6.4* of the Supporting Information for further details about sample preparation and the analytical method.

11.4 Data analysis

Measured $\text{NH}_4\text{-N}$ concentrations (in $\text{mg}\cdot\text{L}^{-1}$) at 1 min^{-1} frequency were averaged over 5 min to obtain a time series that matched the flow data. Subsequently, concentrations were multiplied by flows to obtain loads (in grams) at different time resolutions (5 min, hours, and days). The latter were used to investigate whether particular patterns could be highlighted between different days of the week. Ammonium loads were used as an anthropogenic marker to estimate the size of the population ($P_{\text{NH}_4\text{-N,ISE}}$). A nationwide survey resulted in a population equivalent (PE) of $8.1 \pm 0.37\text{ g}$ of $\text{NH}_4\text{-N}$ per day per individual ($n = 86$ STPs; $R_2 = 0.85$). This study used historic average $\text{NH}_4\text{-N}$ loads and specifically collected census data for STP catchments (see *Section 11.6.10* for further details). However, because of the low industrial input, Lausanne only shows $6.9 \pm 0.4\text{ g}$. Therefore, the latter PE was used to calculate day-specific populations based on $\text{NH}_4\text{-N}$ measurements. All calculations and statistical tests were carried out using Matlab 2012b (The Mathworks, Natick, MA).

11.4.1 Uncertainty assessment

Total uncertainty for the estimated drug target residue (DTR) loads was computed as the square root of the sum of squares of the relative error associated with analysis, flow measurement (i.e., 12.5% according to information provided by the STP personnel), and sampling error (i.e., 15%) (95,102,157). Additionally, THC-COOH concentrations were corrected to account for adsorption onto particulate matter (8% based on findings by Baker et al. (114)). To estimate the uncertainty surrounding the $\text{NH}_4\text{-N}$ in-line measurements ($\text{NH}_4\text{-N}_{\text{ISE}}$), average daily concentrations determined with the ISE were compared to laboratory measurements carried out systematically (between 2 and 4 times a week throughout the year) by the STP personnel ($\text{NH}_4\text{-N}_{\text{Lab}}$). More in detail, daily average $\text{NH}_4\text{-N}$ concentrations were measured in 24h time-proportional composite samples (collected only between Monday and Friday, from 7 a.m. to 7 a.m.) using a spectrophotometer (DR 3900 spectrophotometer, Hach-Lange, Hach Company, Loveland, CO). See *Section 11.6.8* for details about laboratory measurements of $\text{NH}_4\text{-N}$. The corresponding daily average $\text{NH}_4\text{-N}$ concentration determined with the ISE was computed and compared to results from laboratory measurements. On the basis of these findings, a final uncertainty of $\pm 30\%$ (systematic error) was associated with the measurements of the $\text{NH}_4\text{-N}_{\text{ISE}}$ concentration. See *Sections 11.6.5 to 11.6.7* for details about uncertainty calculations.

11.5 Results and discussion

11.5.1 Weekly ammonium patterns

Daily $\text{NH}_4\text{-N}_{\text{ISE}}$ mass loads were computed to determine whether differences between the various days of the week could be highlighted. Results obtained for dry and rainy days are illustrated in Figure 26.

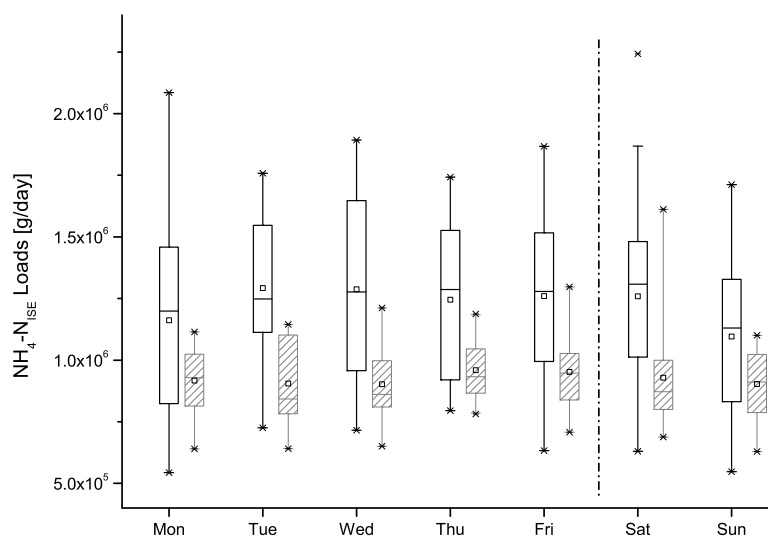


Figure 26: Boxplot of daily $\text{NH}_4\text{-N}_{\text{ISE}}$ loads [$\text{g}\cdot\text{day}^{-1}$] measured during dry (black) and rainy (grey) weather. Monday (dry: $n=23$; wet: $n=13$), Tuesday (dry: $n=25$; wet: $n=11$), Wednesday (dry: $n=20$; wet: $n=16$), Thursday (dry: $n=16$; wet: $n=20$), Friday (dry: $n=21$; wet: $n=15$), Saturday (dry: $n=21$; wet: $n=15$), Sunday (dry: $n=19$; wet: $n=17$)

Days with rain were characterized by lower $\text{NH}_4\text{-N}$ loads and no difference could be observed between the days of the week. This was attributed to the difficulties of the ISE to correctly measure $\text{NH}_4\text{-N}$ concentrations when these were particularly low (i.e., $\leq 1 \text{ mg}\cdot\text{L}^{-1}$), which is the case during heavy rain events because of dilution with runoff water. In contrast, mean loads measured on Mondays and Sundays during dry weather were lower compared to the other days of the week. Loads measured on Sundays were on average 6.5-16% lower than those measured on other days. Statistical analysis showed that the difference between loads measured on Sundays and the other days of the week (for both dry and rainy weather) was significantly lower than zero (left-tailed Wilcoxon signed rank test; p -value < 0.05), except for Mondays where no significant difference could be observed. See Figure 33 of the Supporting Information for an illustration of the measured differences. Official statistics about commuters (150) were used to investigate if these differences could be explained by the contribution of the non-residential population working within the catchment, which is supposed to be less abundant during weekends. The data were used to compute the net increase in the size of the population expected during a week day. However, the available figures date back to 2000, and updated estimates were not available because the procedure to collect population statistics has been modified since and the number of commuters can no longer be estimated (*Service Cantonal de Recherche et d'Information Statistiques*, personal communication). On the basis of the evolution of transportation in the area (230), it was assumed that the ratio between the net number of commuters (i.e., the nonresident population working within the catchment minus the resident population working elsewhere) and the total resident population remained constant or increased but surely did not diminish since 2000. On the basis of these assumptions, an increase of at least 15% of P_{census} (i.e., 34'000 people) was estimated. These results seem to agree with the observations made with $\text{NH}_4\text{-N}_{\text{ISE}}$ loads. According to $\text{NH}_4\text{-N}_{\text{ISE}}$ data, the absence of commuters affected only Sundays and not Saturdays. This could be

linked to the arrival of people for shopping and leisure during Saturdays, because the city represents one of the major urban centers in the region. These findings seem to indicate that intra-weekly fluctuations of $\text{NH}_4\text{-N}_{\text{ISE}}$ reflect the movement of people in and out of the catchment. However, it is not possible to exclude that other unknown factors could contribute to the observed decrease during Sundays.

11.5.2 Ammonium loads and population estimates

Figure 27 illustrates the population size estimates over the investigated period. The average value of $P_{\text{NH}_4\text{-N}_{\text{ISE}}}$ was 190'000 inhabitants (minimum, 78'000; maximum, 479'000; standard deviation, 58'000). In comparison to population estimates based on COD (P_{COD}) and TP (P_{TP}), $P_{\text{NH}_4\text{-N}_{\text{ISE}}}$ yielded lower values (approximately 15%). See Figure 34 of the Supporting Information for a plot of population estimates based on these markers. In the case of population estimates based on $\text{NH}_4\text{-N}_{\text{Lab}}$ ($P_{\text{NH}_4\text{-N}_{\text{Lab}}}$), a good correlation could be established with $P_{\text{NH}_4\text{-N}_{\text{ISE}}}$ (Pearson correlation, 0.73; p -value of $1.25 \times 10^{-10} < \alpha = 0.05$) measured during dry weather, as shown in the scatter plot in Figure 28. During rainy weather, correlation was not so good. As already discussed in the previous section, this is assumed to be linked to the difficulties of the ISE to correctly measure $\text{NH}_4\text{-N}$ concentrations because of dilution from surface runoff. Nonetheless, the average $P_{\text{NH}_4\text{-N}_{\text{ISE}}}$ was in agreement with estimates obtained from laboratory measurements (i.e., average $P_{\text{NH}_4\text{-N}_{\text{Lab}}} = 196'000 \pm 52'000$), and the results from the two techniques were statistically not different (t-test; p -value of $0.65 > \alpha = 0.05$).

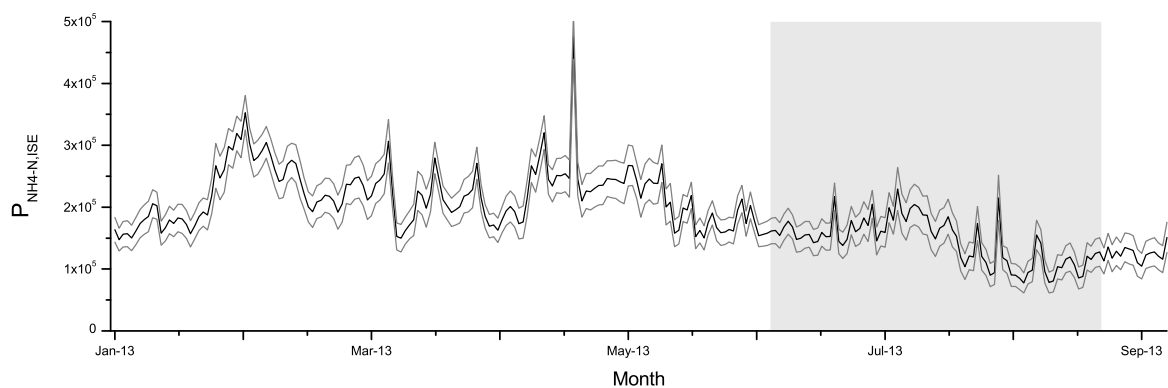


Figure 27: Estimated $P_{\text{NH}_4\text{-N}_{\text{ISE}}}$ (black line) and the associated standard deviation (gray lines). The summer break is indicated by the gray zone between June and September.

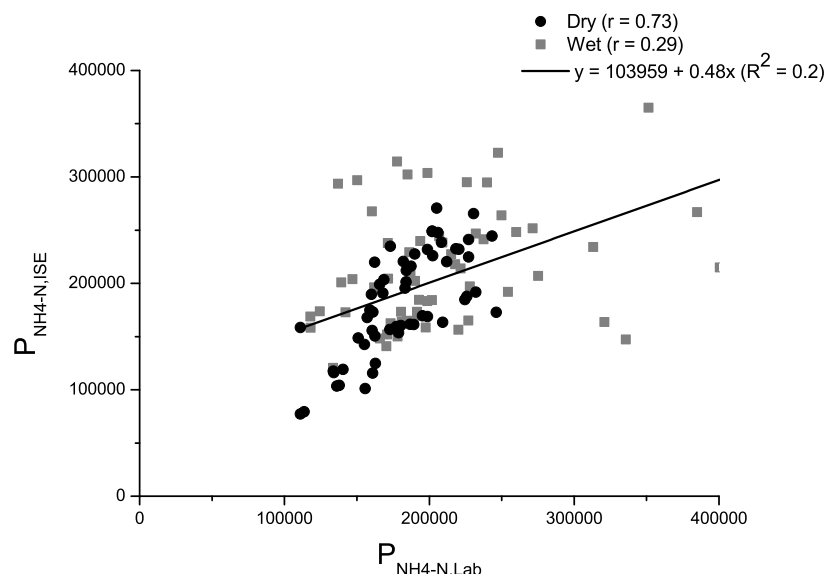


Figure 28: Scatter plot of $P_{\text{NH}_4\text{-N,Lab}}$ versus $P_{\text{NH}_4\text{-N,ISE}}$ measured during dry (black) and rainy (gray) weather. r , Pearson's correlation coefficient; R^2_{adjust} , adjusted coefficient of determination for the best fit line of all data (dry and wet).

Summer months (July and August) were characterized by lower estimates of $P_{\text{NH}_4\text{-N,ISE}}$, as shown in Figure 27. This could be due to summer holidays and the absence of university students. In fact, the STP serves a large university campus with 8'800 employees and 22'300 students (151,152), with the latter being absent during the summer break (from June to September). On the other hand, toward the end of August and the beginning of September, the population tends to increase. This could be due to the end of the summer holidays and the arrival of students for the beginning of the academic year (mid September). As discussed previously, the decrease in $P_{\text{NH}_4\text{-N,ISE}}$ observed on Sundays was between 6.5 and 16% (i.e., 11'500-30'400 inhabitants) compared to other days of the week because of the lower number of commuters. The obtained results show that the fluctuations in $\text{NH}_4\text{-N}$ loads, within the same week or during longer periods of time, reflect changes in the size of the contributing population. Thus, in wastewater-based studies, one could use i) absolute loads of illicit drugs (or any other target compound) or ii) population normalized loads to monitor consumption patterns. The latter can be accomplished i) using raw $\text{NH}_4\text{-N}$ loads and/or ii) using $\text{NH}_4\text{-N}$ population equivalents to estimate the number of people, if site-specific $\text{NH}_4\text{-N}$ population equivalents accounting for non-human contributions are available. These would provide information about population dynamics at different time scales and, thus, help interpret the patterns in illicit drug loads, without additional time-consuming procedures required for the analysis of other anthropogenic markers.

11.5.3 Diurnal patterns of illicit drug and ammonium loads

The diurnal profiles of $\text{NH}_4\text{-N}_{\text{ISE}}$ loads are presented in Figure 29a. Both patterns are characterized by peak concentrations in the morning, in correspondence to highest toilet usage (39,231). During the weekend, the peak was shifted because people tend to wake up later (41) and, as already discussed, measured loads tend to be lower because of limited

contribution of commuters on Sundays. Similar observations were made when investigating diurnal creatinine profiles derived from wastewater analysis (157). During week days (dry weather), two additional peaks were generally visible at 2 p.m. and 8 p.m., which were less pronounced in weekend profiles. These are likely associated with the increased use of toilets during the lunch break and in the evening, when people get back from work.

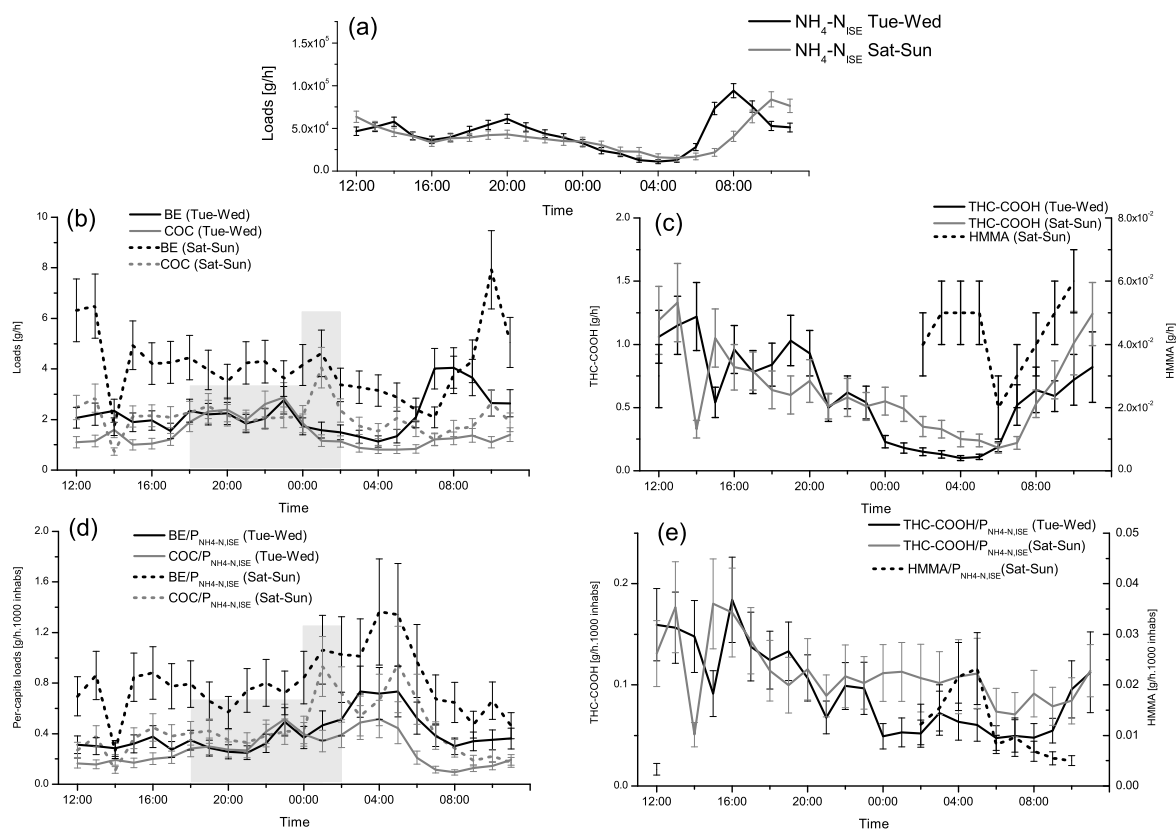


Figure 29: (a) Diurnal profiles of $\text{NH}_4\text{-N}$ measured during the week (Tuesday and Wednesday) and weekend (Saturday and Sunday). (b and c) Absolute and (d and e) $P_{\text{NH}_4\text{-N}_{\text{ISE}}}$ normalized loads for the target compounds measured during the week and the weekend from 12 p.m. to 12 p.m. The gray area in charts b and d indicates the period during which the COC/BE ratio was close or greater than 1.

In the case of BE and COC, higher loads were measured during the weekend (Figure 29b). Interestingly, during the night from Tuesday to Wednesday (area marked in gray in Figure 29b), the COC/BE ratio was larger than 1, although, according to pharmacodynamics, higher concentrations of BE should be observed after consumption of the substance (33). Similarly, between 1 a.m. and 2 a.m. of Sunday morning, a peak in COC loads was observed, while BE loads measured during the same period were below the expected value (COC/BE ratio equal to 0.9 and 0.7). These observations could be due to direct disposal of COC in the sewer system. Analogous findings were reported in previous studies (158,232), and it was suggested that the COC/BE ratio should be interpreted carefully because of various uncertainties associated with excretion and degradation in sewers (158). The mean COC/BE ratio was 0.60 ($n = 48$ hourly + 11 daily samples), which is in the same range as values reported in other studies (158) (i.e., 0.52). BE peak loads were observed during the morning, in concomitance

to the $\text{NH}_4\text{-N}_{\text{ISE}}$ peak. Correlation analysis showed that the two compounds are closely related (cross-correlation between 0.9 and 0.8 for week day and weekend, respectively). On the other hand, the increase in COC during the morning was less pronounced in comparison to the loads measured during the rest of the day. In this case, no particular correlation could be highlighted between COC and $\text{NH}_4\text{-N}_{\text{ISE}}$ (cross-correlation of 0.2 for both days). These findings might indicate that BE is more closely related to the urine content in wastewater compared to COC because it shows more similarities to the classical $\text{NH}_4\text{-N}_{\text{ISE}}$ pattern associated with toilet usage. Similar to what was observed for $\text{NH}_4\text{-N}_{\text{ISE}}$, the increase in BE and COC during the morning appeared later during the weekend compared to the week day. Moreover, loads decreased later during the night of the weekend, indicating that excretion of both BE and COC continued as people stay out for longer periods of time (e.g., nightclubs). This particular pattern supports previous findings about the recreational use of COC (46,157,158). Population normalized loads (i.e., DTR loads divided by $P_{\text{NH}_4\text{-N}_{\text{ISE}}}$), shown in Figure 29d, especially those of BE, showed a nearly constant increase during the evening/night. Of particular interest is the peak at 4-5 a.m., followed by a constant decrease throughout the rest of the morning. Because most of the bars and nightclubs are located in the city center, which is gravity-drained, and the residence time of wastewater from this area is fairly short, this drop could be explained by people going home or leaving the center after closing time (i.e., 5 a.m.). Currently, the municipality of the city is exploring the possibility of modifying the closing time of bars and nightclubs, and it will be interesting to evaluate if this affects the observed patterns.

The presence of MDMA could not unequivocally be confirmed in the analyzed sample because the selected ions for the MRM mode were influenced by the matrix and their ratios were not within the expected ranges. Hence, it was not possible to determine whether the molecule was present or not. In the case of HMMA, this effect was absent and the molecule could be positively identified, as illustrated in Figure 29c. To the knowledge of the authors, this is the first time that a metabolite of MDMA is reported to have been detected in wastewater samples. HMMA could be detected in the first sample from Saturday (12 p.m.) and was then not detectable until 2 a.m. Interestingly, relatively stable absolute loads were observed during the night until 5 a.m. According to pharmacodynamic studies, HMMA is readily detected (0.83 and 3.33 h) in urine after oral administration of MDMA (153). Thus, the observed loads are likely due to a recent consumption that took place during the evening/night. Loads then dropped after 5 a.m., as people tend to go home after closing of nightclubs and bars, already discussed for BE and COC. Subsequently, loads increased constantly to reach the maximum at 10 a.m. Normalized HMMA loads (Figure 29e) were similar to those already observed for BE and showed a steep increase in loads from 2 a.m. to 5 a.m., suggesting an important increase in per-capita excretion during the night, followed by an abrupt decrease between 5 a.m. and 6 a.m.

Absolute THC-COOH loads are illustrated in Figure 29c. In both profiles, THC-COOH loads reached the maximum between 12 p.m. and 2 p.m. Some peaks were observed during the afternoon/evening, and minima were reached during the night. Contrary to what was observed for BE, which reaches its maximum in the morning and then decreases around 9-10 a.m.,

THC-COOH loads continued to increase until the beginning of the afternoon. This could be linked to the metabolism of THC-COOH, which reaches maximum urinary concentrations later compared to BE and COC (6-10h after smoking of cannabis (154,155) versus 5-8h for BE and 4-6h for COC after nasal insufflation of cocaine (156)). However, this could also be due to the non-resident population, which contributes only later to the measured THC-COOH loads. In comparison of the week day and weekend patterns, it can be noticed that THC-COOH loads decrease later during the weekend, similar to what was already observed for BE and COC. On the other hand, an increase in loads is observed during the evening of the week day. During the weekend, the increase in THC-COOH loads in the morning is slightly delayed in comparison to the week day. THC-COOH and $\text{NH}_4\text{-N}_{\text{ISE}}$ loads exhibited a moderate to strong correlation (cross-correlation of 0.6 and 0.8 during the week day and weekend, respectively), suggesting that this metabolite is closely related to the urine content in wastewater. Normalized THC-COOH loads (Figure 29e) were higher during the weekend. In both cases, maximum values were observed in the late afternoon, followed by a general decrease during the evening/night. Some sporadic peaks, in particular, between 10 p.m. and 5 a.m., can be observed in the two patterns. Interestingly, a clear decrease in normalized loads during the night was only visible during the week day, suggesting that excretion of THC-COOH was fairly constant during the night of the weekend. As already discussed for BE and COC, this could be due to the longer activity of people during the weekend nights.

11.5.4 Longitudinal evolution of illicit drug and ammonium loads

Per-capita DTR loads (i.e., grams per day per 1000 inhabitants) were computed with P_{census} and $P_{\text{NH}_4\text{-N,ISE}}$. This was undertaken to determine if the latter could provide additional information to help interpret the observed patterns. Results are illustrated in Figure 30.

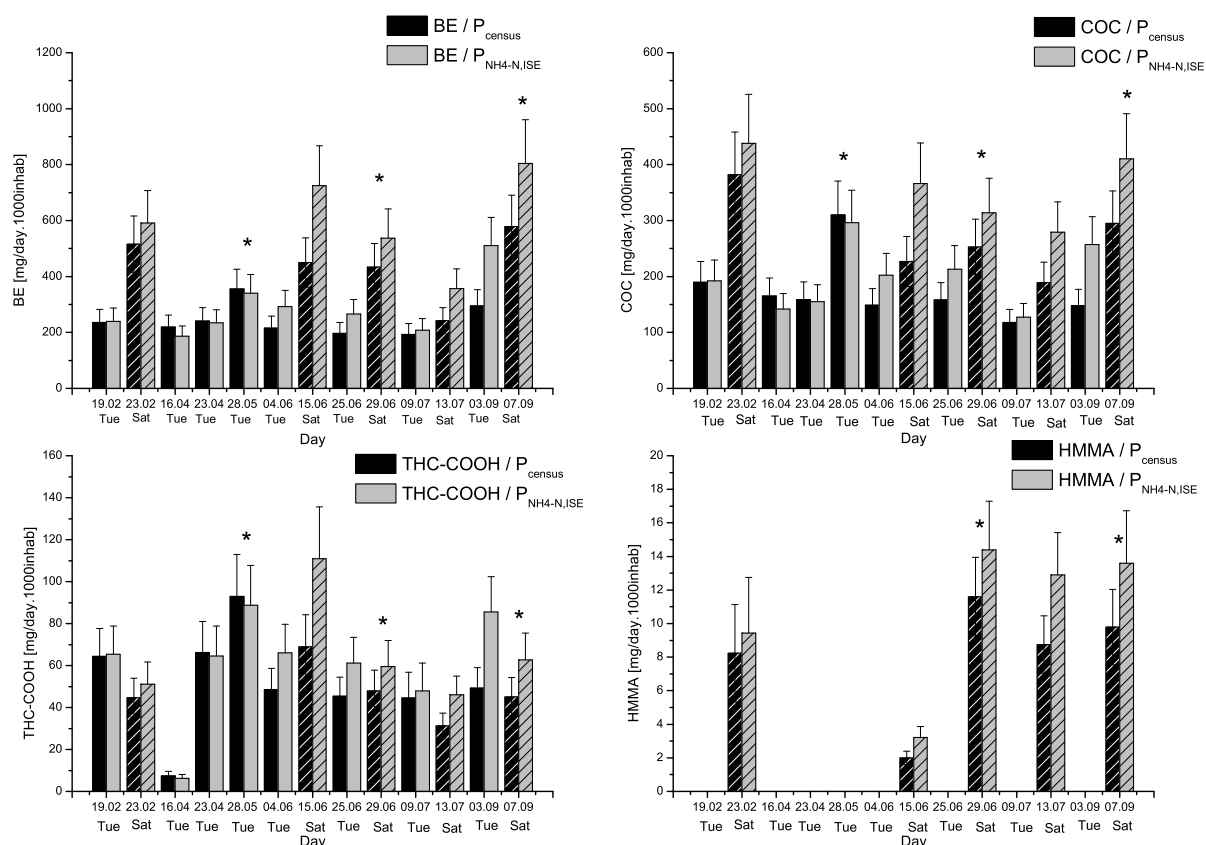


Figure 30: Per-capita loads of DTR (milligrams per day per 1000 inhabitants) based on P_{census} (i.e., 220'000 inhabitants, shown in black) and $P_{\text{NH}_4\text{-N,ISE}}$ (shown in gray). Striped bars show results for Saturdays-Sundays. Days with rain have been marked (*). See Figure 35 of the Supporting Information for the corresponding flows and $\text{NH}_4\text{-N}_{\text{ISE}}$ loads.

COC and BE absolute loads fluctuated between 26 and 83 $\text{g}\cdot\text{day}^{-1}$ and between 43 and 130 $\text{g}\cdot\text{day}^{-1}$, respectively. Within a week, loads measured during the weekend were always higher compared to those measured during the week (Mann-Whitney test; p value of 0.003 and 0.015 $< \alpha = 0.05$ for BE and COC, respectively), in accordance with previous findings (46,157,158). The last data point (i.e., Saturday, September 7) was collected during a major public event (music festival) that took place in the city center. Such events have been associated with an increase in loads because of recreational use of illicit drugs (57,233). In the case of this study, loads were not substantially higher compared to those measured during other weekends. However, this could be due to rainy weather, which negatively affected the presence of people. In comparison to P_{census} , the effect of normalizing with $P_{\text{NH}_4\text{-N,ISE}}$ was to increase the estimated per-capita loads of both BE and COC, in particular, for values measured during the weekends. However, no substantial difference can be observed in the overall patterns, except for loads collected on June 15. A more detailed analysis shows that within a week, the pairwise

difference between loads measured on neighboring Tuesday/Wednesday and Saturday/Sunday was always greater for $P_{\text{NH}_4\text{-N,ISE}}$ compared to P_{census} . However, statistical analysis did not show any significant difference between the two normalization approaches (p -value of 0.1 and $0.17 > \alpha = 0.05$ for BE and COC, respectively). These findings indicate that, in the context of this study, the use of an anthropogenic marker does not substantially modify the overall patterns obtained when investigating census (i.e., static) and $\text{NH}_4\text{-N}$ (i.e., dynamic) normalized illicit drug loads measured in wastewater samples. However, because this indicator is sensitive to fluctuations in the size of the population, it provides more reliable estimates of per-capita loads of illicit drugs excreted or consumed by the investigated population.

As already discussed, the presence of MDMA could not unequivocally be confirmed in the analyzed samples. However, HMMA was detected in the five weekend samples, as shown in Figure 30. The presence of HMMA only during weekends highlights the recreational use of MDMA (158). Measured loads fluctuated between 0.45 and 2.55 $\text{g}\cdot\text{day}^{-1}$. Similar to BE and COC, the effect of normalization with $\text{NH}_4\text{-N}_{\text{ISE}}$ was to increase the estimated per-capita loads because $P_{\text{NH}_4\text{-N,ISE}}$ was below P_{census} during the weekends. Nonetheless, no change in the overall pattern was observed.

Concerning THC-COOH loads, the measured absolute loads were generally between 7 and 20 $\text{g}\cdot\text{day}^{-1}$, except for April 16, which was characterized by particularly low loads (i.e., 1.6 $\text{g}\cdot\text{day}^{-1}$). When measured loads (both absolute and normalized) are observed, it can be seen that, within a week, values recorded during the weekend were systematically lower compared to those measured during the week (3-27%). This seems to contradict previous findings suggesting that cannabis consumption remains constant throughout the week 31 and could be explained by the reduced contribution of the non-resident population, in particular, during Sundays. However, statistical analysis showed no significant difference between loads (absolute and normalized by P_{census} and $P_{\text{NH}_4\text{-N,ISE}}$) measured during the week and the weekend (p -value of $0.34 > \alpha = 0.05$). Additional samples will have to be collected to obtain a larger data set and to better assess these findings. Similar to what was already observed for BE and COC, no substantial difference can be observed in the overall pattern of P_{census} and $P_{\text{NH}_4\text{-N,ISE}}$ normalized loads. This was confirmed by comparing the pairwise difference of loads measured on consecutive Tuesday/Wednesday and Saturday/Sunday (p -value of $0.9 > \alpha = 0.05$) and suggest, as for BE and COC, that normalization with this particular anthropogenic marker does not affect the overall pattern but allows for more precise determination of the per capita loads of illicit drugs excreted or consumed by the investigated population.

11.5.5 Implications and outlooks

Online measurements of $\text{NH}_4\text{-N}$ were shown to be sensitive to weekly (i.e., commuters) and seasonal (i.e., summer holidays) fluctuations in the size of the population. Within a week, changes of 11'500-30'400 inhabitants could be detected and associated with smaller contribution of the non-resident population during Sundays. Overall, the estimated size of the population contributing to the generated wastewater was below values derived from census

data and other water-quality parameters. These findings illustrate that the use of a human-specific marker is necessary to objectively estimate the size of the contributing population in sewage epidemiological studies.

The investigated substances showed a distinct pattern during the weekend, which could be associated with the prolonged activities of people during the night compared to week days. In the particular case of BE, COC, and HMMA, normalization with $\text{NH}_4\text{-N}$ showed that loads tend to increase during the night from Saturday to Sunday and then suddenly drop after 5 a.m., likely because of the closing of bars and nightclubs. BE and THC-COOH showed a strong correlation with $\text{NH}_4\text{-N}_{\text{ISE}}$ loads, which supports the validity of the marker to interpret fluctuations of these particular metabolites. Additionally, these findings suggest that high-resolution $\text{NH}_4\text{-N}_{\text{ISE}}$ data could be used to extrapolate BE and THC-COOH loads. However, the collection of additional 24h samples is necessary to evaluate the feasibility of this approach.

In this context, normalization with $P_{\text{NH}_4\text{-N,ISE}}$ generally increased the estimated per-capita loads compared to P_{census} , in particular, during weekends because of the smaller size of the population. This suggests that, in this particular catchment, census data and water-quality parameters, such as COD, TN, and TP, tend to underestimate the per-capita loads of illicit drug residues reaching the STP. Interestingly, the overall pattern for the investigated illicit drug residues did not change substantially between P_{census} and $P_{\text{NH}_4\text{-N,ISE}}$ normalized loads. This suggests that the magnitude of fluctuations in the size of the population is negligible compared to that of illicit drug consumption. However, substantial changes in the size of the population could occur in longitudinal studies, during longer sampling campaigns or major events. In this situations, the use of an anthropogenic marker, such as $\text{NH}_4\text{-N}$, could be extremely useful to account for these changes, because these would not be discernible when using per-capita loads obtained with a static or non-human specific marker.

The results obtained in the context of this work showed that $\text{NH}_4\text{-N}$ can be used as an anthropogenic marker to monitor fluctuations in the size of the population in wastewater-based epidemiological studies. This can be achieved by back-calculating a number of inhabitants (using population equivalents of $\text{NH}_4\text{-N}$) or, if the objective is to monitor consumption in a specific area, using raw $\text{NH}_4\text{-N}$ loads as an indirect marker of the population. By assuming that non-human sources are constant, the latter can be used when no information about the contribution of external sources is available for the investigated catchment. Moreover, normalizing DTR loads with an anthropogenic marker overcome uncertainties because of systematic errors in flow measurements (138).

11.6 Supporting Information

11.6.1 Sampling campaign

Details about the sampling day, type of sample and the collection period are reported in Table 17.

Table 17: Details of the sampling campaign. Eleven daily 24-hour composites and 48 hourly samples were collected.

<i>Date</i>	<i>Weekday</i>	<i>Sample Type</i>	<i>Sampling Time</i>
19.02.13	Tuesday	Daily 24-hour composite	12PM-12PM
23.02.13	Saturday	Daily 24-hour composite	12PM-12PM
16.04.13	Tuesday	Daily 24-hour composite	12PM-12PM
23.04.13	Tuesday	24 hourly samples	12PM-12PM
28.05.13	Tuesday	Daily 24-hour composite	12PM-12PM
04.06.13	Tuesday	Daily 24-hour composite	12PM-12PM
15.06.13	Saturday	24 hourly samples	12PM-12PM
25.06.13	Tuesday	Daily 24-hour composite	12PM-12PM
29.06.13	Saturday	Daily 24-hour composite	12PM-12PM
09.07.13	Tuesday	Daily 24-hour composite	12PM-12PM
13.07.13	Saturday	Daily 24-hour composite	12PM-12PM
03.09.13	Tuesday	Daily 24-hour composite	12PM-12PM
07.09.13	Saturday	Daily 24-hour composite	12PM-12PM

11.6.2 Standards and chemicals

Standards of (\pm)-3,4-methylenedioxymethamphetamine (MDMA), 4-hydroxy-3-methoxymethamphetamine (HMMA), cocaine (COC), benzoyecgonine (BE) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) were purchased from Lipomed (Lipomed AG, Arlsheim, Switzerland) as 1 mg.mL⁻¹ solutions in methanol. Deuterated analogues of (\pm)-3,4-methylenedioxymethamphetamine-d₃ (MDMA-d₃), cocaine-d₃ (COC-d₃), benzoyecgonine-d₃ (BE-d₃) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH-d₃) were purchased from Lipomed (Lipomed AG, Arlsheim, Switzerland) as 0.1 mg.mL⁻¹ solutions in methanol. Deuterated analogue of 4-hydroxy-3-methoxymethamphetamine-d₃ (HMMA-d₃) was purchased from ReseaChem (ReseaChem GmbH, Burgdorf, Switzerland) in powder form (10 mg) and reconstituted in methanol at 0.1 mg.mL⁻¹. Stock and work solutions were prepared in methanol (MeOH) and stored in the dark at -20°C. Methanol, ethyl acetate, ammonium hydroxyde (25%), hydrochloric acid (37%) and

N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) were analytical grade and purchased from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland).

11.6.3 Sample preparation

Immediately after collection, wastewater samples were filtered (0.7 μm nitrocellulose filters, type GF/F Whatman®, GE Healthcare), acidified to pH 2 using 37% hydrochloric acid and spiked with deuterated standards (100 $\text{ng}\cdot\text{L}^{-1}$). Subsequently, samples were extracted using Oasis MCX (Waters, Milford, MA, USA) solid-phase extraction (SPE) cartridges (3cc, 60mg). Wastewater samples (500 mL) were loaded at 5 $\text{mL}\cdot\text{min}^{-1}$ and cartridges were then dried with nitrogen. If analysis could not be carried out immediately, cartridges were wrapped in aluminium foil and stored at -20°C . Elution was carried out with 2.5mL of methanol and 2.5mL of methanol with 5% of NH_4OH . The eluate was evaporated to dryness and reconstituted in 100 μL of Ethyl Acetate and 100 μL of MSTFA and heated at 80°C for 60 min.

11.6.4 Instrumental analysis

Analyses were carried out on an Agilent 7890A Gas Chromatograph coupled to an Agilent 7000B Triple-Quadrupole mass spectrometer (Agilent, Santa Clara, CA, USA). Separation was achieved on a HP-5 MS (5% phenyl methyl siloxane) capillary column (20 m length, 0.18 mm internal diameter and 0.18 μm film thickness) with a column flow of 0.9 $\text{mL}\cdot\text{min}^{-1}$. Analyses were carried out in the MRM mode and for each compound and its corresponding deuterated standard, three transitions were monitored when possible (target ion for quantitation and two qualifier ions for confirmation). Calibration was achieved by injecting standard solutions of target analytes (0.5, 5, 25, 50, 250, 500 and 1000 $\text{ng}\cdot\text{mL}^{-1}$) and the corresponding deuterated analogues (100 $\text{ng}\cdot\text{mL}^{-1}$). Four control standards at different concentrations (2.5, 37.5, 125 and 625 $\text{ng}\cdot\text{mL}^{-1}$) were analysed at regular intervals and were considered acceptable if the RSD was $\leq 20\%$. Method detection (MDL) and method quantification limit (MLQ) were determined by spiking blank MilliQ water samples with increasing amounts of the target compounds and by extracting them using the previously described SPE method. MDL and MLQ were defined as the lowest analyte concentration giving a signal-to-noise (S/N) ratio ≥ 3 and ≥ 10 , respectively. Intermediary precision for the entire analytical procedure was determined by spiking blank MilliQ water samples at three different concentrations (i.e., 20, 50 and 100 $\text{ng}\cdot\text{L}^{-1}$). These were extracted and analysed in triplicates during three different days (3 samples for each day). Table 18 summarizes the parameters of the analytical method.

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Table 18: Summary of analytical parameters and recoveries for the target compounds. R_t : Retention Time; IS: Internal Standard; T: Target ion for quantitation; Q_1 & Q_2 : Qualifier ions for confirmation; MQL: Method quantification limit.

Target Compound	R_t	IS	MRM Transition (m/z)	MQL [ng.L ⁻¹]	Intermediary Precision	Recovery (RSD %) (n = 24)
MDMA	5.3	MDMA-d ₃	T: 129.9 → 73.0	1	4.6%	69.5% (8.5%)
			Q1: 129.9 → 45.1			
			Q2: 129.9 → 58.0			
HMMA	6.2	HMMA-d ₃	T: 129.9 → 73.0	3	4.9%	74.3% (7.9%)
			Q1: 129.9 → 45.1			
			Q2: 129.9 → 58.0			
COC	8.5	COC-d ₃	181.8 → 82.0	1	16.6%	73.6% (5.3%)
			181.8 → 91.0			
			181.8 → 65.0			
BE	8.7	BE-d ₃	239.8 → 82.0	5	5.1%	72.7% (5.9%)
			239.8 → 91.0			
			239.8 → 65.0			
THC-COOH	11.2	THC-COOH-d ₃	473 → 355.2	4	19.4%	48.7% (9.2%)

11.6.5 Equation S1

Calculation of the uncertainty associated to NH₄-N_{ISE} loads measurement:

$$RE_{NH_4-N_{Loads}} = \sqrt{(RE_{NH_4-N_{Conc}})^2 + (RE_Q)^2}$$

where $RE_{NH_4-N_{Loads}}$, $RE_{NH_4-N_{Conc}}$ and RE_Q are the relative errors associated to the calculated NH₄-N_{ISE} loads (in grams per time unit), the measured NH₄-N_{ISE} concentration (in mg.L⁻¹) and flow (in litres per time unit) at the inlet of the STP, respectively.

11.6.6 Equation S2

Calculation of the uncertainty associated to the estimation of illicit drug loads in the collected samples:

$$RE_{DTR} = \sqrt{(RE_{Sampling})^2 + (RE_{Analysis})^2 + (RE_Q)^2}$$

where RE_{DTR} is the error associated to the measured loads (in g.day⁻¹), $RE_{Sampling}$ and $RE_{Analysis}$ are the errors associated to the sampling and the analytical method.

11.6.7 Equation S3

Calculation of the uncertainty associated to the estimation of illicit drug loads normalised by population estimates derived from the NH₄-N_{ISE}:

$$RE_{DTR_{Norm}} = \sqrt{(RE_{NH_4-N, Conc})^2 + (RE_{Sampling})^2 + (RE_{Analysis})^2}$$

where $RE_{DTR_{Norm}}$ is the relative error associated to the $P_{NH_4-N, Conc}$ normalized DTR loads.

11.6.8 Laboratory NH_4-N analyses

NH_4-N_{Lab} measurements were carried out with a DR3900 Spectrophotometer (Hach Lange, Hach Company, Loveland, CO, USA) using LCK303® ammonium cuvettes and ADDISTA® multi parameter standard solutions for quantitation and quality assurance (Hach Lange, Hach Company, Loveland, CO, USA). According to practice standards in the STP's laboratory, instrument maintenance is carried out once a year. Proficiency tests, organized by A.G.L.A.E. (Hallennes lez Haubourdin, France), are carried out on a yearly base. The latest tests was successfully passed in September 2013 (expected NH_4-N value: 13.98 $mg.L^{-1}$; measured NH_4-N value: $14.5 \pm 0.1 mg.L^{-1}$).

11.6.9 Extent of urea hydrolysis in the collected wastewater samples

To determine the extent of hydrolysis of urea in samples collected at the entrance of the STP, a grab sample (1L) was collected and immediately (t_0) analyzed to determine the NH_4-N concentration. It was decided not to collect a 24h composite sample to avoid storage and hydrolysis which could occur during the sampling period. The same sample was then analyzed after 24 (t_{24h}), 48 (t_{48h}) and 72 hours (t_{72h}) to measured the NH_4-N content. The sample was stored in the dark at room temperature (20°C). Analyses were carried out using the laboratory equipment described above. Results are reported in Table 19:

Table 19: Measured NH_4-N concentrations immediately after collection (t_0) and after 24 (t_{24h}), 48 (t_{48h}) and 72 (t_{72h}) hours. The results for the reference (QC) standard are reported in the last column

<i>Sample</i>	<i>NH_4-N Concentration [$mg.L^{-1}$]</i>	<i>ADDISTA® reference standard (expected concentration 25 $mg.L^{-1}$)</i>
t_0	48.7	24.9
t_{24h}	47.6	25.0
t_{48h}	46.0	24.9
t_{72h}	45.8	24.8

No substantial difference in NH_4-N concentration was measured after 24, 48 and 72 hours, suggesting that urea is completely hydrolyzed the time it reaches the inlet of the STP.

11.6.10 NH_4-N Population estimates

The data used to compute population estimates based on measured NH_4-N_{ISE} loads was derived from a Swiss survey conducted between 2003 and 2006 in 86 sewage treatment plants (STP) to evaluate the mass fluxes of pollutants, amongst which was NH_4-N (234). Using linear regression on the mean values measured between 2003 and 2006, a population equivalent (PE) of $8.1 \pm 0.4 g.day^{-1}.inhabitant^{-1}$ ($R^2 = 0.85$) of NH_4-N was estimated. The STP

investigated in the context of this study was not among those considered in the aforementioned survey. Furthermore, the estimated PE was obtained considering STP which are likely to be more affected by industrial and agricultural contribution. In this catchment, such contributions are known to be limited as the STP serves mainly a residential area and services from tertiary sector. Thus, using average daily $\text{NH}_4\text{-N}$ loads measured by the STP and the number of inhabitants during at the time the survey was conducted (i.e., 2003–2006) (235–237), a PE specific for this catchment was computed. All details from the survey and the regression analysis are reported in Figure 31, Table 20 and Table 21.

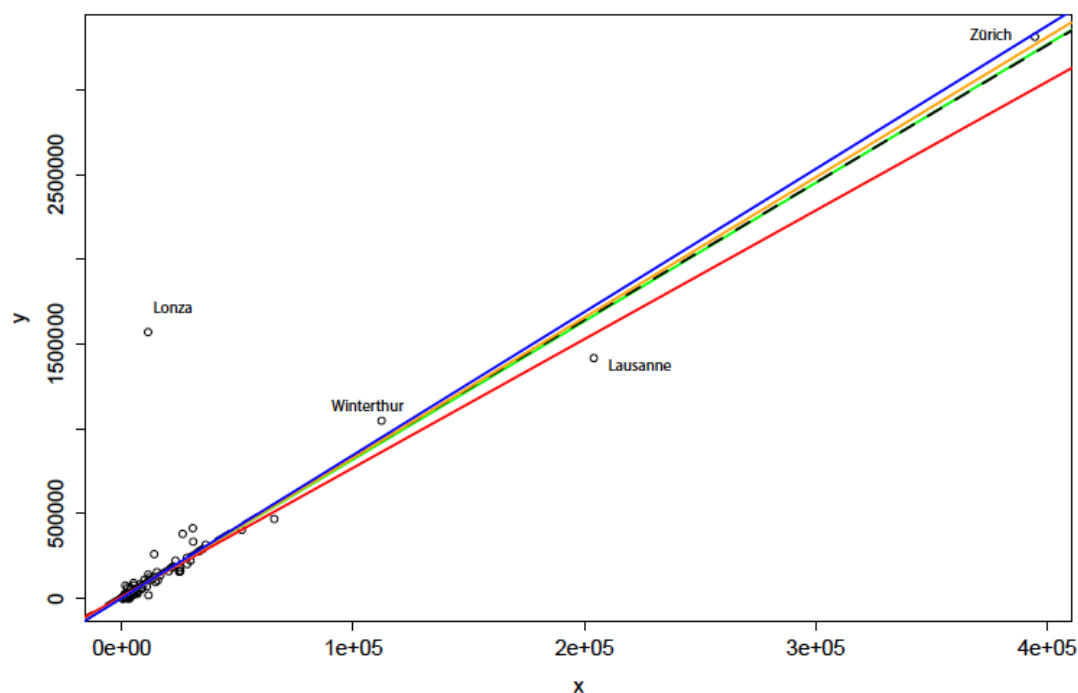


Figure 31: Regression analysis of the $\text{NH}_4\text{-N}$ data from the Swiss survey for the period 2003-2006. *x-axis*: inhabitants, *y-axis*: yearly average $\text{NH}_4\text{-N}$ ammonium loads. See Table 20 for a description of the regressions.

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Table 20: Summary of the parameters used for the regression analysis on NH₄-N data gathered in the Swiss survey for the period 2003-2006.

<i>WWTPs</i>	<i>Color of regression line</i>	<i>NH₄-N PE [g.day⁻¹.inhab⁻¹]</i>	<i>Standard Error</i>	<i>R²</i>
All STP				
Including the catchment investigated in the context of this work (i.e., Lausanne, yearly average NH ₄ -N loads = 1.42x10 ⁶ g.day ⁻¹ , number of inhabitants = 204080) n=86	Black dashed	8.10	0.37	0.85
Excluded Lonza				
(Outlier, STP heavily influenced by industry) n=85	Green	8.15	0.11	0.98
Excluding Lonza and Zurich-Werdhölzli (biggest STP determining the regression) n = 84				
Excluding Lonza, Werdhölzli, Lausanne and Winterthur (Outlier and the three biggest STP in the regression) n = 82	Orange	8.26	0.34	0.88
Excluding Lonza and Lausanne (Outlier and large STP with lower NH ₄ -N loads than average) n = 84	Blue	8.43	0.10	0.99

The results confirm that this particular catchment is below the national average in terms non-human sources of NH₄-N. Analogous figures are observable for data from 2007 and 2008 (238,239), confirming the results from 2003-2007. Additionally, the obtained value corresponds to the PE commonly used by the STP personnel, i.e. 7 g.day⁻¹.inhabitant⁻¹ of NH₄-N (personal communication (240)). Thus, to account for the lower industrial/agricultural contribution in this particular catchment, and by considering that no major industrial plant or agricultural area has been connected to the system, the estimated value of 6.9 ± 0.4 g.day⁻¹.inhabitant⁻¹ was used to compute size of the population based on online measurements of NH₄-N.

Table 21: Summary of the data used to estimate the catchment specific PE. The last row contains the mean values for the period 2003-2006 which were used to compute the final PE used in the context of this study. a) Mean population provided by the STP for the period 2003-2006.

<i>Year</i>	<i>Mean NH₄-N Loads [g/d]</i>	<i>Inhabitants (Census)</i>	<i>Inhabitants (PE = 8.1 ± 0.4 [g.day⁻¹.inhab⁻¹])</i>	<i>Difference (%)</i>	<i>Expected PE [g.day⁻¹.inhab⁻¹]</i>
2003	1444842	198687	178375	11%	7.3
2004	1470955	203720	181599	11%	7.2
2005	N/A	N/A	N/A	N/A	N/A
2006	1337778	206268	165157	20%	6.5
Mean: 2003-2006	1417858	204080^a	175044	14%	6.9 (0.4)

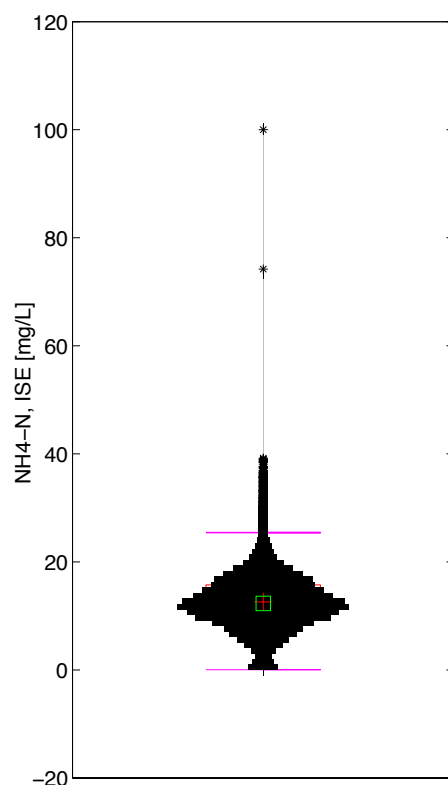


Figure 32: Distribution of the NH₄-N concentration measured with the ISE during the sampling campaign. Average concentration: 12.6 ± 5.4 mg.L⁻¹.

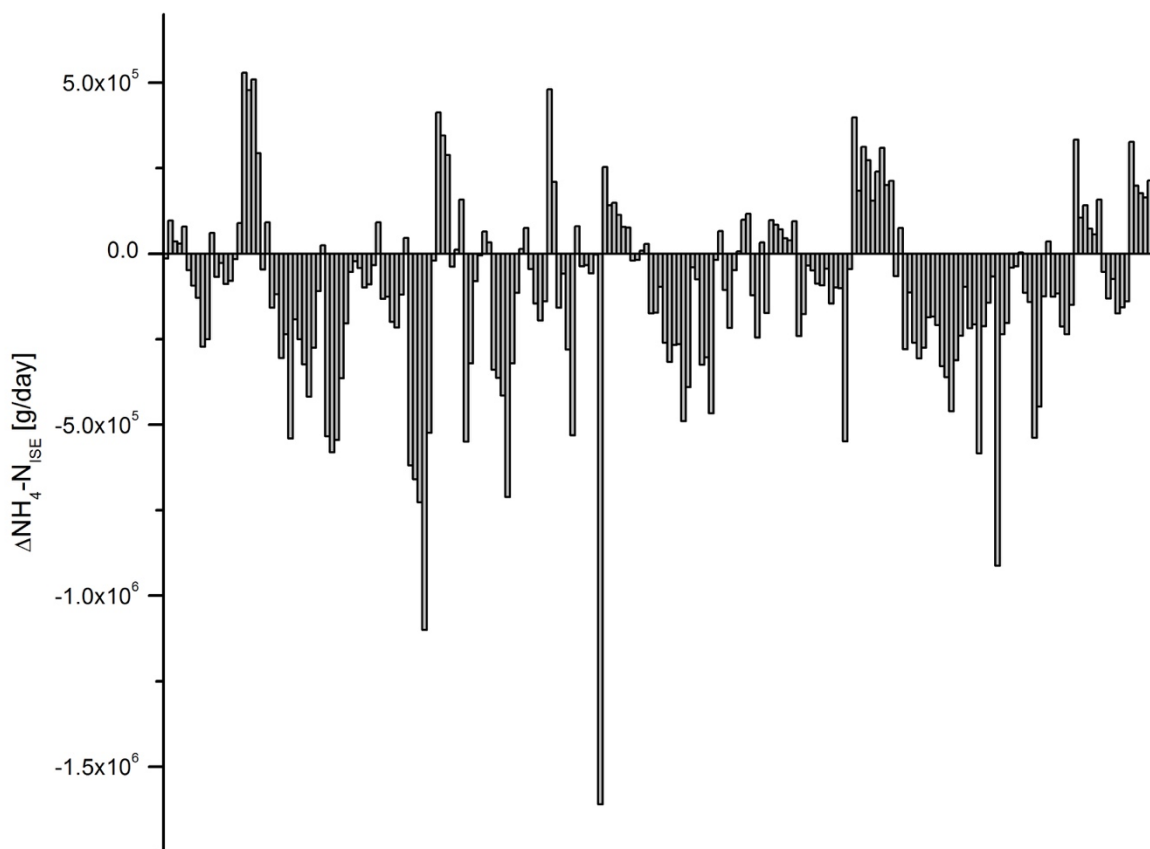


Figure 33: Illustration of the differences in $\text{NH}_4\text{-N}_{\text{ISE}}$ loads [$\text{g}\cdot\text{day}^{-1}$] measured between Sundays and the other days of the week throughout the sampling campaign.

11.6.11 Population estimates with other pollutants:

Measurements of Chemical oxygen demand ($120 \text{ g}\cdot\text{day}^{-1}\cdot\text{inhab}^{-1}$) (241), total phosphorous ($1.6 \text{ g}\cdot\text{day}^{-1}\cdot\text{inhab}^{-1}$) (231) and $\text{NH}_4\text{-N}$ carried out during the year by the STP personnel were used to estimate the size of the population. The obtained time series are plotted below.

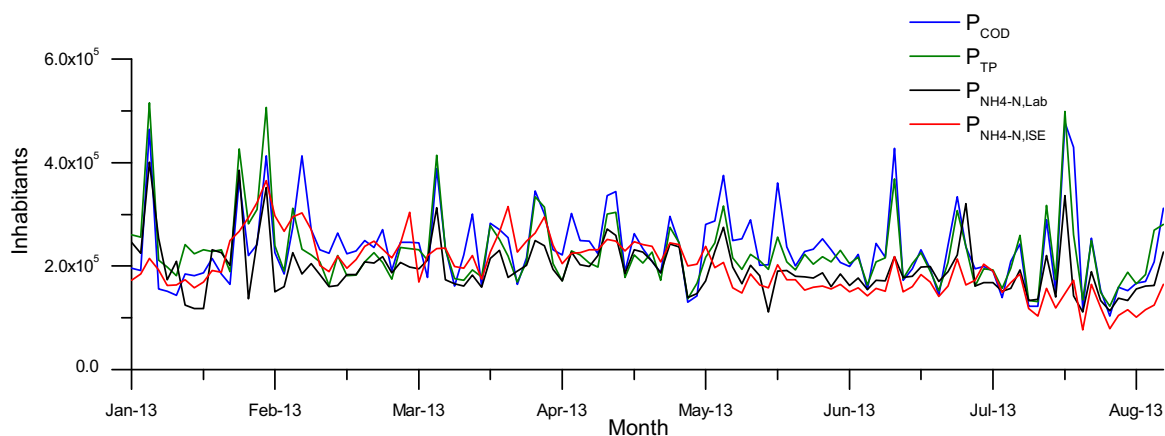


Figure 34: Estimated size of the population based on chemical oxygen demand (P_{COD}), total phosphorus (P_{TP}), laboratory measurements of $\text{NH}_4\text{-N}$ ($P_{\text{NH}_4\text{-N,Lab}}$) and $\text{NH}_4\text{-N}$ measurements obtained with the ISE ($P_{\text{NH}_4\text{-N,ISE}}$) during the investigated period.

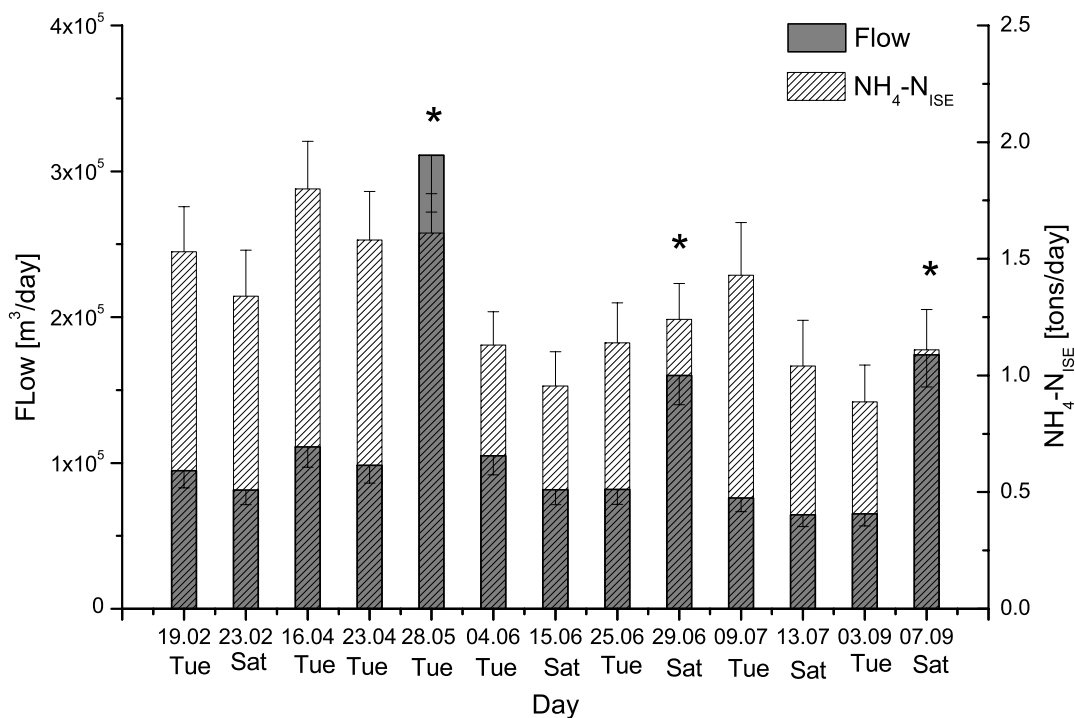


Figure 35: Flows and NH₄-N_{ISE} loads measured during the sampling campaign. Days with rain have been marked with (*).

XII Annexe II: Data triangulation in the context of opioid monitoring via wastewater analyses

Been F., Benaglia L., Lucia S., Gervasoni J-P., Esseiva P., Delémont O., *Data Triangulation in the Context of Opioid Monitoring via Wastewater Analyses*, Drug and Alcohol Dependence, 2015, 151, 203-210; <http://dx.doi.org/10.1016/j.drugalcdep.2015.03.022>

12.1 Abstract

Background: The need to contextualize wastewater-based figures about illicit drug consumption by comparing them with other indicators has been stressed by numerous studies. The objective of the present study was to further investigate the possibility of combining wastewater data to conventional statistics to assess the reliability of the former method and obtain a more balanced picture of illicit drug consumption in the investigated area. **Methods:** Wastewater samples were collected between October 2013 and July 2014 in the metropolitan area of Lausanne (226'000 inhabitants), Switzerland. Methadone, its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), the exclusive metabolite of heroin, 6-monoacetylmorphine (6-MAM), and morphine loads were used to estimate the amounts of methadone and heroin consumed. **Results:** Methadone consumption estimated from EDDP was in agreement with the expectations. Heroin estimates based on 6-MAM loads were inconsistent. Estimates obtained from morphine loads, combined to prescription/sales data, were in agreement with figures derived from syringe distribution data and general population surveys. **Conclusions:** The results obtained for methadone allowed assessing the reliability of the selected sampling strategy, supporting its ability to capture the consumption of a small cohort (i.e., 743 patients). Using morphine as marker, in combination with prescription/sales data, estimates in accordance with other indicators about heroin use were obtained. Combining different sources of data allowed strengthening the results and suggested that the different indicators (i.e., administration route, average dosage and number of consumers) contribute to depict a realistic representation of the phenomenon in the investigated area. Heroin consumption was estimated to approximately 13 g.day⁻¹ (118 g.day⁻¹ at street level).

Keywords: wastewater, illicit drugs, epidemiology, triangulation, methadone, heroin.

12.1 Introduction

Wastewater-based epidemiology has been presented in the literature as an additional tool to monitor illicit drug consumption in a given community. Numerous researches have been conducted providing valuable data about the amounts of drug residues found in wastewater. These data emphasize geographical and time variabilities regarding the types and amounts

of metabolites that were detected in wastewaters (135,136). But the significance and the meaning of these data are still an issue to be questioned. Researchers and actors directly interested by the understanding and monitoring of drug consumption, while acknowledging the information gained through these data, have stressed the need to contextualize wastewater-based data and to combine it to epidemiological data (159,160), allowing to assess the relevance and complementarity of the indicators and to obtain a more informed picture of the phenomenon (159). So far, wastewater-based estimates have generally been compared to national figures and the number of studies which have focused on a detailed comparison of epidemiological and sewage data are limited (242,243). Heroin consumption is a known problem in Switzerland, which culminated in an open drug scene at the beginning of the 1990's (244). Numerous harm reduction, prevention and follow up programmes have been set up since. These provide a unique source of information about injecting drug users (IDU), in particular heroin users, and their consumption habits. Based on these valuable sources of information, the present study addresses the problem of opiate consumption by focusing on the combined perspectives provided by general population surveys, opioid substitution therapy, needle and syringe distribution programmes and wastewater analyses. As illustrated in Figure 36, the first objective of the present work was to test the reliability of the methodology by comparing estimates of methadone consumption derived from wastewater analyses to figures obtained from the registry of people undergoing opioid substitution therapy as well as from prescriptions of methadone for pain treatment.

The second objective consisted in evaluating the feasibility of using 6-monoacetylmorphine (an exclusive metabolite of heroin) loads measured in wastewater samples to estimate and monitor heroin consumption. For this, estimates were compared to figures derived from morphine (major metabolite of heroin) loads. However, as shown in Figure 36, morphine is not an exclusive metabolite of heroin consumption and its presence in wastewater is also due to the consumption of other (licit) products (35,116). Thus, it was necessary to include prescription and sales data of licit products. The final objective consisted in combining results from wastewater analyses to other sources of information to study their complementarity and obtain a more informed picture of the extent of heroin consumption in the investigated area, as shown in Figure 36. Because wastewater analyses provide quantitative and chronological data, focus was set on sources of information from which comparable (quantitative) data could be inferred (see Figure 36).

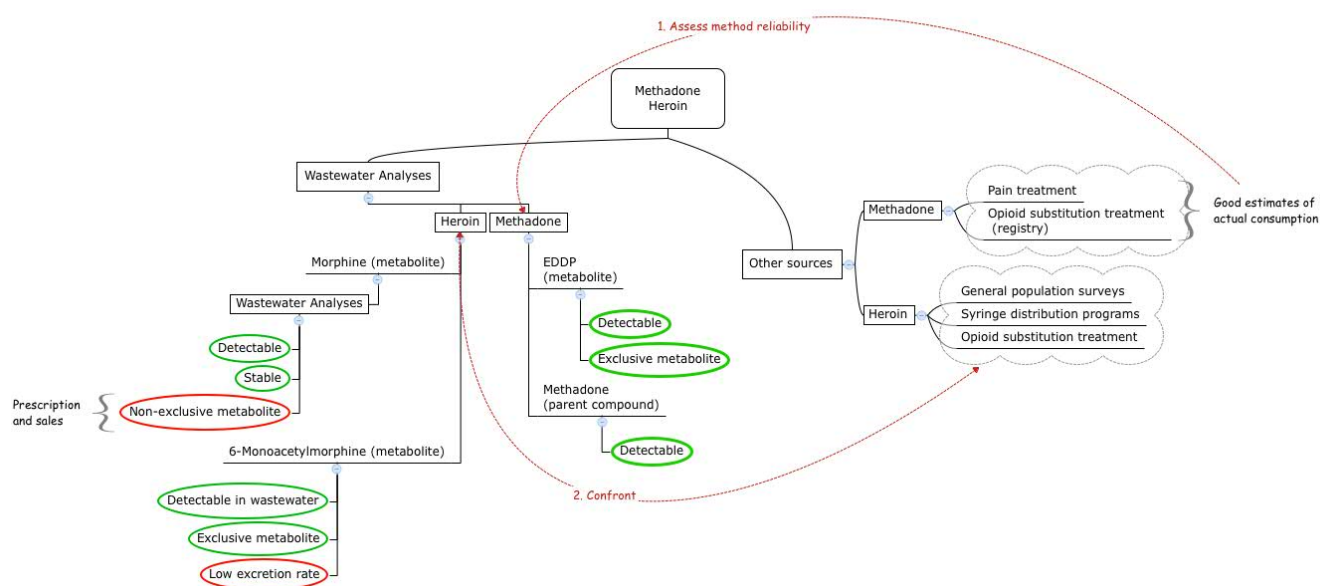


Figure 36: Scheme of the available data sources for methadone, heroin and morphine and the two objectives of the study.

12.2 Methods

12.2.1 Target domain, wastewater sampling and analysis

The present research focuses on the city of Lausanne (Switzerland) and its metropolitan area (city plus 15 additional municipalities), with a population of 226,000 inhabitants (census based) served by a unique sewage treatment plant (STP). As far as possible, samples were collected every second week between October 2013 and July 2014. The sampling approach used, extensively described in (76) can be summarized as follows; briefly, volume-proportional composite samples were collected from Tuesdays to Wednesdays and from Saturdays to Sundays, from 12PM to 12PM, for a total of 28 samples. The following substances were targeted: methadone (MET) and its major metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), the exclusive metabolite of heroin, 6-monoacetylmorphine (6-MAM), and morphine (MOR).

After solid-phase extraction (Oasis MCX, 3cc, 60mg, Waters, Milford, MA, USA), samples were analysed with a UHPLC system (1290 Infinity, Agilent, Santa Clara, CA, USA) coupled to a triple quadrupole mass spectrometer (5500 QTrap, ABSciex, Ontario, Canada). The entire analytical method was previously validated. A summary of the validation results is reported in Table 22 (see Supporting Information for further details).

Table 22: Summary of validation results. Accuracy and precision were evaluated in triplicates at three concentrations in spiked drinking water. Recovery was calculated in wastewater samples (n=3). Method detection limit (MDL) was determined by spiking blank water samples with increasing analyte concentrations. The MDL was set as the lowest analyte concentration giving a signal-to-noise of three and a correct ratio between the signals of the monitored ions. See Supporting Information for more details.

	<i>Concentration [ng.L⁻¹]</i>	<i>Accuracy</i>	<i>Precision</i>	<i>R²</i>	<i>MDL [ng.L⁻¹]</i>	<i>%Recovery (SD)</i>
MOR	50	23.3%	3.7%	0.9987	2	89.7 (2.2)
	250	13.2%	8.6%			
	1250	9.5%	9.4%			
6-MAM	50	14.5%	12.2%	0.9981	5	85.6 (55.1)
	250	8.9%	8.1%			
	1250	9.9%	9.6%			
EDDP	50	15.6%	5.5%	0.9998	5	75.5 (8.1)
	250	13.2%	5.9%			
	1250	6.4%	8.7%			
MET	50	10.9%	5.6%	0.9997	0.5	88.2 (3.6)
	250	11.0%	7.3%			
	1250	0.6%	12.7%			

12.2.2 Data analysis and back-calculations

Using a similar strategy as suggested by Jones et al. (147), substance loads (concentrations multiplied by daily wastewater flow), back-calculations (quantity of parent drug initially consumed, based on measured loads and excretion data) and the associated errors were computed using Monte Carlo simulations. All parameters are reported in Table 23.

Table 23: Parameters used for Monte Carlo simulations. a) Percentage of initial dose excreted as target compound; b) Ratio between the amounts of target compound adsorbed onto suspended matter and dissolved in the aqueous phase; c) Excretion after administration of heroin only; d) Excretion after administration of morphine only. SE = Standard Error

	<i>Source</i>	μ	<i>SE</i>	<i>Distribution</i>	<i>Note</i>
Flow	Initial data provided by the STP in $\text{m}^3 \cdot \text{s}^{-1}$ averaged over 5min ($n = 288$ measurements per day). Sum to obtain daily flow in $\text{L} \cdot \text{day}^{-1}$ from 12 p.m. to 12 p.m.	Daily flow [$\text{L} \cdot \text{day}^{-1}$]	Residuals from flow regression using a Gaussian model	Normal (μ, SE^2)	See Supporting Information for further details.
Chemical Analysis	Result of chemical analysis and associated analytical error.	Mean concentration of 3 replicates [$\text{ng} \cdot \text{L}^{-1}$]	$\frac{SD}{\sqrt{3}}$	Normal (μ, SE^2)	Standard deviation derived from the validation of the analytical method and the analysis of 3 replicates
	(164)	EDDP: 25.0%	EDDP: 4.6%	Beta (a, b)	Data derived from the tables available in (164), i.e. 6 individuals submitted to 3 experiments (low, intermediate and high dosage)
		MET: 19.7%	MET: 2.6%	Beta (a, b)	
Excretion^f		6-MAM: 0.46%	6-MAM: 0.09%	Beta (a, b)	
		MOR ^c : 48.0%	MOR ^c : 2.4%	Beta (a, b)	Inverse-variance weighted average
		MOR ^d : 74.6%	MOR ^d : 1.6%	Beta (a, b)	
	Data derived from literature review by (35)	6-MAM: 0.6%	6-MAM: 0.11%	Beta (a, b)	Inverse-variance weighted average of studies focused only on intravenous and intramuscular administration
		MOR ^c : 53.4%	MOR ^c : 2.8%	Beta (a, b)	
Adsorption^p	Used data from (114,165,166)	EDDP: 23.5%	EDDP: 5.9%	Normal (μ, SE^2)	Inverse-variance weighted average
		MET: 12.1%	MET: 2.1%	Normal (μ, SE^2)	
	Used data from (165,166)	6-MAM: na	6-MAM: na	na	
		MOR: 4.1%	MOR: 1.1%	Normal (μ, SE^2)	Inverse-variance weighted average

Based on stability profiles reported by Chen et al. (112) after addition of $\text{Na}_2\text{S}_2\text{O}_5$, and because sample processing was carried out within 2h after collection of the last sub-sample, degradation onsite and during transport were assumed to be negligible. Additionally, a systematic sampling error of $\pm 15\%$ was considered (76,95,102,157). Simulations and statistical analyses were performed with Matlab R2012b (The MathWorks Inc., Natick, MA, USA).

12.2.3 Methadone supplies and prescription data

Two sources were used to estimate consumption: (i) total methadone supplies for pharmacies, hospitals and physicians in Canton Vaud (no catchment specific data was available) between October 1st 2013 and June 30th 2014 (161) and (ii) an anonymised registry of all patients undergoing opioid substitution therapy with methadone in Canton Vaud (162). Of the 1632 patients undergoing substitution therapy, 1492 were administered methadone while the

remaining were treated with other pharmaceuticals (e.g., Subutex®, Temgesic®, Ketalgine®). The amount of methadone consumed per day by patients was estimated as follows:

$$\mathbf{Methadone}_{OSTM} = \sum_{i=1}^n \mathbf{u}_i * \mathbf{d}_i \quad \mathbf{Equation\ 1}$$

were u_i is the unitary dose ($\text{mg}\cdot\text{dose}^{-1}$) and d_i is the number of daily doses to be taken by the i^{th} patient in the database. To select patients relevant for the area under investigation, two criteria were used: i) residence of the patient or that of the responsible physician were within the catchment and ii) only patients registered as active at the beginning of the sampling were considered.

12.2.4 Heroin and morphine data

To obtain quantitative estimates about heroin consumption, to be compared with data derived from wastewater analyses, the number of syringes distributed per month by specialized centres within the catchment was used (163). Amongst the low threshold facilities disseminated on the territory, four were considered for this study. Distribution by pharmacies was also included. However, no updated monthly registry was available for the investigated period. Based on figures derived from a survey conducted in all pharmacies of the region in 2011 (245), it was estimated that 26% of all syringes are distributed by pharmacies (see Supporting Information). Heroin consumption based on distributed syringes was estimated as follows:

$$\mathbf{Heroin\ Syringes}(u_i) = n * inj * u_i \quad \mathbf{Equation\ 2}$$

where n is the number of distributed syringes and inj is the proportion of heroin users for whom injection is the major administration route. Based on a study conducted in the investigated area, only 52.6% of heroin users ($n = 148$) reported injection as the major administration route (171). u_i is the unitary dose reported in the literature, i.e. $100 \text{ mg}\cdot\text{dose}^{-1}$ (172), $30 \text{ mg}\cdot\text{dose}^{-1}$ (145) and the midpoint between the reported dosages ($65 \text{ mg}\cdot\text{dose}^{-1}$). It was assumed that syringes are used only once as, according to data from 2011, only 4.9% of IDU attending low threshold facilities reported to have injected with used syringes in the last month (16). Finally, it was considered that the distributed syringes are used within the catchment area and that syringe usage is homogeneously distributed throughout the month. Clearly these assumptions have not been verified and will be discussed further on.

Analogous to methadone prescription data, monthly morphine supplies in Canton Vaud for the period between October 1st 2013 and June 30th 2014 were provided by the authorities (161). Data for preparations containing other substances, which are partially metabolised to morphine after consumption (i.e., codeine, pholcodine, ethylmorphine and nicomorphine (35)), were derived from monthly sell-in made by physicians, pharmacies and hospitals in Canton Vaud (246). Daily consumptions were estimated by assuming negligible stocks and homogeneous consumption throughout the month. In combination with pharmacokinetic data,

morphine loads derived from the consumption of these pharmaceuticals ($L_{\text{Morphine,Pharm}}$) were computed.

12.3 Results and discussion

12.3.1 Methadone supplies and substitution treatment registry

Based on supply data, 3.3 kg of methadone (base form) were distributed each month in Canton Vaud during the investigated period. By assuming that all methadone distributed is consumed and that stocks are negligible, it was estimated that approximately 116 grams are used per day. The amount distributed for substitution therapy was estimated to 100.3 g.day^{-1} , which corresponds to 86.3% of the total, as reported in Table 24. Even though methadone is mainly used for opioid substitution therapy, it is also used for treatment of severe pain (247,248) (see Figure 36), which explains the difference between the two. The criteria described previously (see *Section 12.2.3*) were used to identify substitution therapy patients, which are likely to consume in the catchment area. A total of 743 people were retained and the daily methadone consumption was estimated to 58.4 g.day^{-1} , which makes up for 58.2% of all methadone distributed for OSTM. Based on these results, and because catchment-specific supply data was not available, it was assumed that methadone used in the catchment for OSTM represents only 86.3% of the total consumption of methadone in the catchment. Thus, total consumption in the catchment was estimated to 67.7 g.day^{-1} , as shown in Table 24.

Table 24: Total MET supplies and quantities distributed for opioid substitution treatment with methadone (OSTM) in the whole region and in the catchment.

<i>Regional data</i>		
Total MET Supply [g.day^{-1}]	116.3	
OSTM	Total for OSTM [g.day^{-1}]	100.3
	OSTM / Total	86.3%
<i>Catchment data</i>		
OSTM [g.day^{-1}]	58.4	
Estimated total MET supply [g.day^{-1}]	67.7	

12.3.2 Wastewater analysis – Methadone

Loads of methadone and its metabolite, EDDP, ($L_{\text{MET,WW}}$, $L_{\text{EDDP,WW}}$) measured during the sampling campaign are shown in Figure 37. A slight increase in $L_{\text{EDDP,WW}}$ is visible towards March and April, but no particular trend is visible, suggesting that methadone consumption is stable throughout time. Moreover, no significant difference was observed between samples collected during the week and during the weekend (Wilcoxon rank sum test p -value = 0.76 and $0.34 > \alpha = 0.05$ for MET and EDDP, respectively). In one occasion, a significantly higher $L_{\text{MET,WW}}$ was measured (see Figure 37), which could be due to direct disposal of methadone, and was considered as an outlier (Grubb's test, $\alpha = 0.05$).

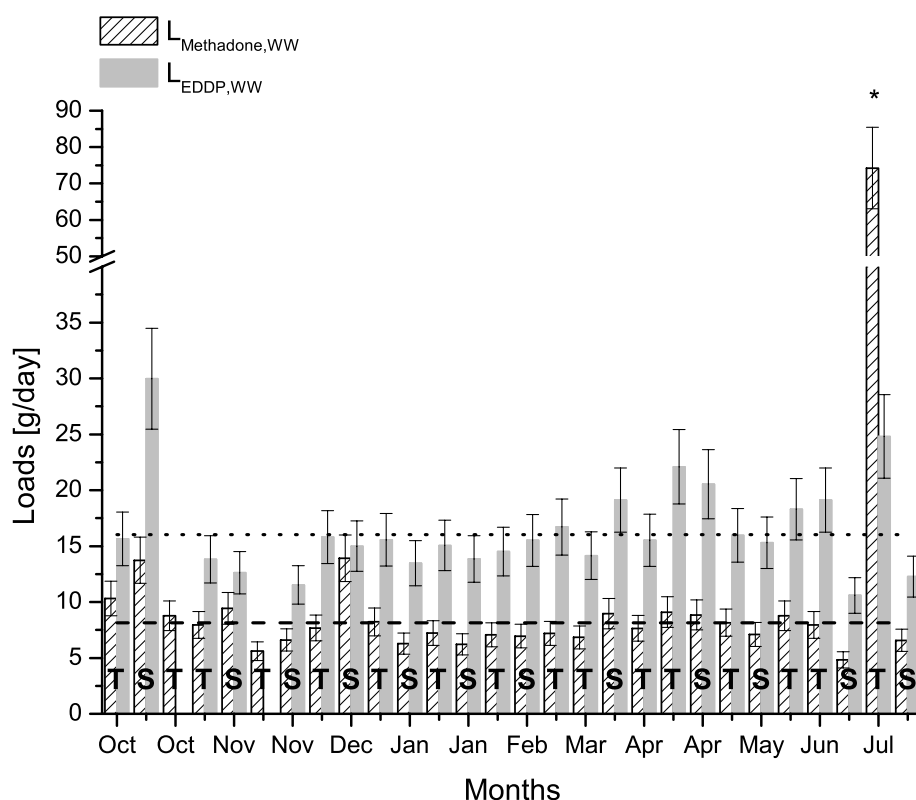


Figure 37: Methadone ($L_{\text{Methadone,WW}}$) and EDDP ($L_{\text{EDDP,WW}}$) loads measured during the sampling campaign (no adsorption correction). The dotted and dashed lines represent the mean loads (after exclusion of the outliers) for EDDP and methadone, respectively. T = Sample collected from Tuesday to Wednesday, S = Sample collected from Saturday to Sunday. (*) $L_{\text{MET,WW}}$ outlier.

Generally, $L_{\text{Methadone,WW}}$ were lower compared to $L_{\text{EDDP,WW}}$, which is in agreement with previous findings (158). The ratio of EDDP to methadone was equal to 1.95 ± 0.45 , which is similar to results obtained in urinalysis from patients undergoing chronic substitution therapy (1.8-2.2) (167,168). Using the previously described parameters, Monte Carlo simulations were performed to back-calculate the amount of methadone initially consumed. Outliers were excluded prior to calculations and results are shown in Table 25.

Table 25: Mean methadone consumption estimated via Monte Carlo simulations with EDDP and methadone loads ($L_{\text{EDDP,WW}}$, $L_{\text{Methadone,WW}}$) and estimates obtained from supplies data. Reported estimates were computed after exclusion of outliers and without considering adsorption onto particulate matter.

	<i>Methadone [g.day⁻¹]</i>		
	<i>Wastewater analyses</i>		<i>Supplies</i>
	$L_{\text{EDDP,WW}}$ (n = 24)	$L_{\text{Methadone,WW}}$ (n = 26)	
Mean	75.6	41.7	67.7
SD	19.3	8.7	-

Simulations were performed with and without accounting for adsorption on suspended solids. This was made because addition of $\text{Na}_2\text{S}_2\text{O}_5$ (added to empty bottles prior to sampling) substantially decreases the pH of the collected wastewater samples (pH = 6.2). At such values, both EDDP and MET are protonated and their distribution coefficient are substantially lower ($\log D = 1.72$ and 1.82 at pH 6.2 for EDDP and methadone, respectively) compared to untreated wastewater ($\log D = 2.73$ and 3.01 at pH 7.5) (249). Thus, the proportion adsorbed is likely negligible in this context and correcting for it could tend to overestimate total loads. The results obtained with and without considering adsorption were in the same order of magnitude as estimates from supplies data. For the sake of clarity, only estimates obtained without considering absorption are reported here (see Table 31 in the Supporting Information for more details). Results obtained with $L_{\text{Methadone,WW}}$ are globally lower, likely due to the higher intra- and inter-individual variability in excretion of methadone compared EDDP (167–169). The good agreement between expected and measured loads, suggest that there are no additional sources (legal or not) of methadone. This is consistent with previous findings (170), where it was shown that very few people in the investigated catchment acquire methadone through alternative (illicit) channels, implying a limited diverted or parallel distribution. Another important aspect is that the obtained results can be used to assess the reliability of the methodology. The consistency between wastewater measurements and expectations suggests that the sampling strategy is suitable to capture relevant wastewater pulses (containing the target compounds) generated by a small cohort (95). With such premises, the sampling strategy should allow the monitoring of substances used by similar (e.g., heroin) or supposedly larger (e.g., cannabis and cocaine) populations. Moreover, the results suggest that there are no apparent losses, degradations or other unknown phenomena, which occur in the sewer system.

12.3.3 Syringe distribution estimates – Heroin

From January 2009 until June 2014, $10'188 \pm 2141$ syringes were distributed on average each month (See Figure 40 in *Section 12.6.5* for a plot of syringe distribution). A steep drop was observed between the end of 2012 and mid 2013, caused by extraordinary police activities aimed at countering the illicit drug market. Since then, the number of distributed syringes has been increasing again. For the period considered in this context, 9925 ± 1062 syringes were distributed. By adding the 26% expected to be distributed by pharmacies, the monthly average for the investigated period is equal to $12'508 \pm 1339$. This corresponds to 411 ± 44 syringes per day. Using the assumptions made previously (see *Section 12.2.4*), it was estimated that between 6.5 ± 0.6 and 21.6 ± 2.0 g.day^{-1} (midpoint 14.1 ± 1.3 g.day^{-1}) of pure heroin are consumed per day, depending on the considered dosage (i.e., 30, 100 or 65 mg.syringe^{-1}).

12.3.4 Morphine supplies and sell-in data

To extrapolate catchment specific daily per capita consumption of morphine from supplies data, two criteria were used: i) the size of the population and ii) the number of hospital beds. The latter was used because nearly 50% of all hospital beds in Canton Vaud are within the catchment (250) and because it is assumed that an important proportion of morphine is

administered in hospitals. The midpoint between estimates obtained according to the two criteria was used to calculate morphine loads generated by the consumption of pharmaceuticals ($L_{\text{Morphine,Pharm}}$). Pharmacies sell-in data were used to estimate the contribution to $L_{\text{Morphine,Pharm}}$ due to the consumption of preparations containing codeine pholcodine and ethylmorphine. Because there was no catchment specific data, estimates were extrapolated based on the size of the population. For hospitals sell-in data, the number of beds was used to calculate catchment specific estimates. Total $L_{\text{Morphine,Pharm}}$ are reported in Table 26. Data for nicomorphine was not available; but as only one product is available on the Swiss market (82), its contribution to $L_{\text{Morphine,Pharm}}$ is likely in the same order of magnitude as pholcodine and ethylmorphine and was thus considered negligible. Average $L_{\text{Morphine,Pharm}}$ generated by the consumption of the considered pharmaceuticals was estimated to $15 \pm 0.5 \text{ g.day}^{-1}$ (range 5.8 – 21.1).

Table 26: Estimated daily average morphine loads due to consumption of pharmaceuticals containing morphine (MOR), codeine (COD), pholcodine (PHO) and ethylmorphine (ETH) in the catchment (161,246). a) Proportion of COD excreted as MOR $6.51 \pm 0.56\%$ (data taken from (35)); b) Proportion of PHO excreted as MOR 0.75% (data taken from (35), standard deviation was not reported); c) Proportion of ETH excreted as MOR $15.3 \pm 2.73\%$ (data taken from (35)).

		<i>Values in [g.day⁻¹]</i>									
<i>Month</i>		<i>Oct-13</i>	<i>Nov-13</i>	<i>Dec-13</i>	<i>Jan-14</i>	<i>Feb-14</i>	<i>Mar-14</i>	<i>Apr-14</i>	<i>May-14</i>	<i>Jun-14</i>	<i>Mean</i>
MOR	Mean	11.8	12.5	15.8	0.9	5.2	17.0	3.0	13.4	14.8	10.5
	SD	0.2	0.3	0.3	0.0	0.1	0.4	0.1	0.3	0.3	0.2
MOR (COD)^a	Mean	4.6	5.0	5.3	4.9	5.1	4.0	4.3	3.6	3.6	4.5
	SD	0.4	0.4	0.5	0.4	0.4	0.3	0.4	0.3	0.3	0.4
MOR (PHO)^b	Mean	0.0018	0.0017	0.0021	0.0017	0.0022	0.0019	0.0014	0.0011	0.0009	0.0016
	SD	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	N/A
MOR (ETH)^c	Mean	0.0007	0.0008	0.0008	0.0007	0.0008	0.0006	0.0007	0.0005	0.0005	0.0007
	SD	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Total MOR	Sum	16.4	17.5	21.1	5.8	10.3	21.0	7.4	17.1	18.4	15.0
	SD	0.5	0.5	0.6	0.4	0.5	0.5	0.4	0.4	0.4	0.5

12.3.5 Wastewater analysis – Heroin and Morphine

6-monoacetylmorphine and morphine loads ($L_{6\text{-MAM,WW}}$, $L_{\text{Morphine,WW}}$) measured in wastewater during the sampling campaign are shown in Figure 38. In agreement with previous studies (56,158,251), no particular trend could be detected the 6-monoacetylmorphine loads ($L_{6\text{-MAM,WW}}$). Significantly high values were measured in three occasions (see (*) in Figure 38), which were identified as outliers (Grubbs Test, $\alpha = 0.05$). The presence of such extreme values could be due to direct disposal of heroin in sewers, which has been shown to be extremely unstable and rapidly undergoes hydrolysis to 6-monoacetylmorphine (6-MAM) and morphine (112,121). After excluding these, the average daily $L_{6\text{-MAM,WW}}$ was equal to $1.84 \pm 0.29 \text{ g.day}^{-1}$.

Morphine loads measured during the end of January were particularly low, while a nearly two-fold increase was observed in samples from the following months. Similar observations were made when taking into account the possible fluctuations in the size of the population (see Figure 41 in the Supporting Information for further details). One of the collected samples

exhibited significantly higher morphine loads than the average (see (#) in Figure 38), and was considered as an outlier (Grubbs Test, $\alpha = 0.05$). Its occurrence could also be due to direct disposal. After exclusion of the extreme value, the estimated average daily $L_{\text{Morphine,WW}}$ was equal to $19.0 \pm 3.1 \text{ g}\cdot\text{day}^{-1}$.

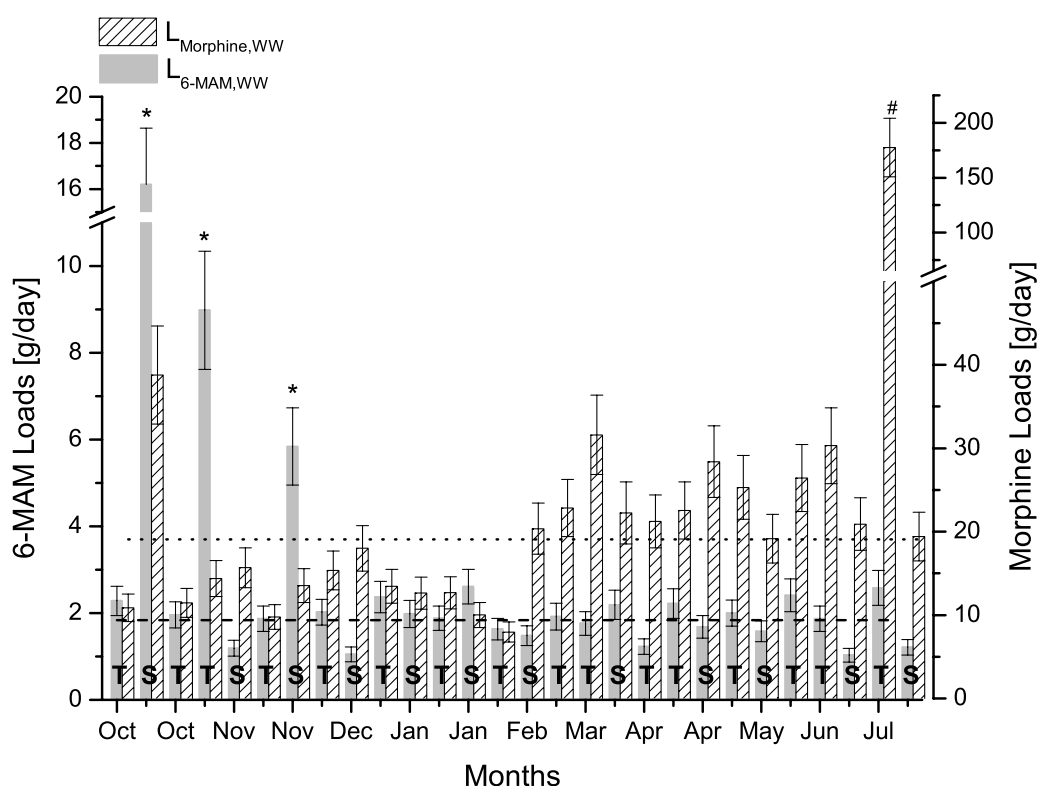


Figure 38: Morphine and 6-monoacetylmorphine loads ($L_{\text{Morphine,WW}}$, $L_{\text{6-MAM,WW}}$), not corrected for adsorption, measured during the sampling campaign. The dotted and dashed lines represent the mean loads (after exclusion of the outliers) for $L_{\text{Morphine,WW}}$ and $L_{\text{6-MAM,WW}}$, respectively. T = Sample collected from Tuesday to Wednesday, S = Sample collected from Saturday to Sunday. (#) $L_{\text{Morphine,WW}}$ outlier, (*) $L_{\text{6-MAM,WW}}$ outliers.

From measurements of 6-MAM and morphine loads, simulations were made to estimate heroin consumption (see Table 27). In the case of morphine, adsorption onto particulate matter was assumed to be negligible due to the decrease in pH after addition of $\text{Na}_2\text{S}_2\text{O}_5$ ($\log D = -1.11$ at pH 6.2 and $\log D = 0.08$ at pH 7.5 (249)). It is however interesting to emphasize that there was only a 4% increase in estimated morphine loads when adsorption was included in the calculations (see Table 32 in the Supporting Information). To the authors' knowledge, no data is available about adsorption of 6-MAM onto particulate matter. Heroin consumption estimates based on $L_{\text{6-MAM,WW}}$ are extremely high and most likely not plausible. Considering that the average purity of heroin street samples in the investigated area is 11% this would correspond to a consumption of $4.3 \text{ kg}\cdot\text{day}^{-1}$. For a population of 226'000, such an estimate is unreasonably high. In the case of morphine-based estimates, if one assumes that there are no other sources of morphine than heroin, the daily consumption would correspond to 51.6-53.8 grams of pure heroin, or approximately $479 \text{ g}\cdot\text{day}^{-1}$ (at 11% purity). Although

overestimated, this value is substantially below estimates derived from $L_{6\text{-MAM,WW}}$ and supports the inconsistency of figures obtained with 6-MAM.

Table 27: Amount of pure heroin consumed in the catchment estimated from 6-MAM and morphine ($L_{6\text{-MAM,WW}}$, $L_{\text{Morphine,WW}}$) loads using Monte Carlo simulations. Reported estimates were computed after exclusion of outliers and without considering adsorption onto particulate matter.

<i>Heroin [g.day⁻¹]</i>		
	$L_{6\text{-MAM,WW}}$ (n=24)	$L_{\text{Morphine,WW}}$ (n=26)
Mean	542.1	51.6
SD	163.4	8.9

Its occurrence in wastewater has been reported only in a limited number of studies (52,56,57,158,251). In two cases, the authors reported $L_{6\text{-MAM,WW}}$ of 2.5-3.75 g.day⁻¹ (values derived from Figure 2 in (56)) for 780'000 inhabitants (56) and 3.25 g.day⁻¹ for 1'100'000 inhabitants (158). After back-calculation, this would correspond to a daily consumption of 600 to 900 g.day⁻¹ of pure heroin. In one of these studies (56), the authors also reported morphine loads ranging from 78 to 86 g.day⁻¹. Back-calculations of heroin consumption based on these figures would give a daily consumption of 190-210 g.day⁻¹, which is again an overestimate but still substantially lower compared to estimates based on 6-MAM. Concentrations of the latter compound measured in this context (7.4-82.4 ng.L⁻¹, mean 23.5 ng.L⁻¹), are similar to results from a previous study conducted in 5 major sewage treatment plants in Switzerland (between limit of quantitation and 82 ng.L⁻¹, mean 38 ng.L⁻¹) (57). The difficulty with obtaining realistic and reliable estimates when 6-MAM is used as indicator for heroin consumption could be linked to the limited number of relevant pharmacodynamics studies (116) as well as its low stability in wastewater (86,114,121,123). Administration routes other than injection (e.g., fumigation and nasal insufflation) could also influence the excretion rates and, thus, bias the obtained estimates. Furthermore, 6-MAM is already present in street samples (175,176) and, according to a small study of street samples analysed by our laboratory (n = 15), the median proportion of 6-MAM to heroin is 10% (unpublished data). Its occurrence in street samples could have an impact on urinary excretion. Finally, elimination via faeces, which has not been reported yet, could also have an impact on the measured loads (35).

Because 6-MAM does not provide realistic figures about heroin consumption, an attempt was made to estimate it using morphine loads measured in the collected wastewater samples. However, as discussed above, it is necessary to account for the contribution from consumption of pharmaceuticals to avoid overestimations. Thus, the difference ($\Delta L_{\text{Morphine}}$) between morphine loads measured in wastewater ($L_{\text{Morphine,WW}}$) and loads expected in wastewater due to the consumption of pharmaceuticals ($L_{\text{Morphine,Pharm}}$) was computed. Because morphine is excreted also after consumption of pharmaceuticals other than morphine (see Table 23), these calculations were performed using morphine loads (measured in wastewater or estimated from prescription/sell-in data) instead of consumed amounts of parent compound (i.e., back-calculation). For $L_{\text{Morphine,WW}}$, samples collected during the same month were averaged to obtain a single value which could be compared to $L_{\text{Morphine,Pharm}}$. In three instances, the

difference between $L_{\text{Morphine,WW}}$ and $L_{\text{Morphine,Pharm}}$ was not statistically significant (t-Test, p -value > 0.05 , see Figure 39).

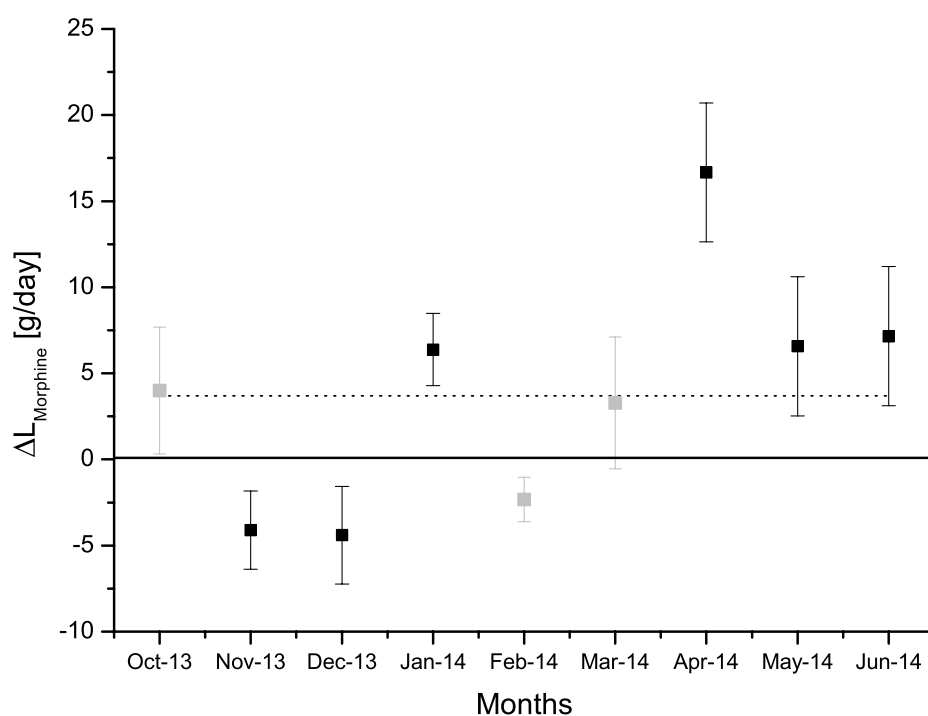


Figure 39: Difference between $L_{\text{Morphine,WW}}$ and $L_{\text{Morphine,Pharm}}$. For each month, the average $L_{\text{Morphine,WW}}$ was calculated and the obtained value was subtracted to $L_{\text{Morphine,Pharm}}$ for the corresponding month. Non-significant differences are shown in gray. The dotted line represents the average $\Delta L_{\text{Morphine}}$.

Negative $\Delta L_{\text{Morphine}}$ occurred when loads derived from wastewater analyses were lower than those calculated from prescription/sales data. This shows the importance of collecting samples repeatedly throughout time, since isolated samples are likely to provide a biased picture of the situation. Average $L_{\text{Morphine,WW}}$, $L_{\text{Morphine,Pharm}}$ and $\Delta L_{\text{Morphine}}$ calculated over the entire sampling campaign are reported in Table 28. Based on $\Delta L_{\text{Morphine}}$, it was estimated that $12.7 \text{ g}\cdot\text{day}^{-1}$ of pure heroin are consumed on average (corresponding to $115.5 \text{ g}\cdot\text{day}^{-1}$ of street purity). Important standard deviations were obtained due to the fluctuations in both $L_{\text{Morphine,WW}}$ and $L_{\text{Morphine,Pharm}}$. Nonetheless, the estimates are in the same order of magnitude than figures obtained from syringe distribution data, as shown in Table 28. A corroborating perspective is provided by figures derived from the latest general population survey: in 2013, the yearly prevalence of heroin consumption in Switzerland was equal to 0.1% (15 to 75 and more years of age) (173), which related to the size of the catchment area, would correspond to 193 individuals (see Supporting Information for further details). Using the dosages indicated previously and by assuming that the considered cohort consumes one dose per day, the estimated daily consumption would be $5.8\text{-}19.3 \text{ g}\cdot\text{day}^{-1}$ of pure heroin, as shown in Table 28.

Table 28: The first part of the table reports morphine loads measured in wastewater ($L_{\text{Morphine,WW}}$) and expected from consumption of pharmaceuticals, as calculated in Table 26 ($L_{\text{Morphine,Pharm}}$). The difference ($\Delta L_{\text{Morphine}}$) was calculated by subtracting $L_{\text{Morphine,Pharm}}$ from $L_{\text{Morphine,WW}}$. The second part of the table reports the estimated heroin consumption back-calculated from $\Delta L_{\text{Morphine}}$ (which is assumed to be excreted after heroin consumption), from syringe distribution data and general population surveys (using three dosages). a) The mean reported here was calculated from the average $L_{\text{Morphine,WW}}$ computed for each month. The standard deviation was calculated as the square root of the sum of squares of the standard deviations of each month.

<i>Morphine loads</i> <i>[g.day⁻¹]</i>		
^a Measured in wastewater ($L_{\text{Morphine,WW}}$) (SD)		19.1 (3.1)
Estimated from prescription and sales data ($L_{\text{Morphine,Pharm}}$) (SD)		15.0 (0.5)
Difference ($\Delta L_{\text{Morphine}}$) (SD)		4.7 (6.5)
<i>Estimated heroin consumption</i> <i>[g.day⁻¹]</i>		
$\Delta L_{\text{Morphine}}$ (SD)		12.7 (8.3)
	Dose [mg]	
	30	6.5
Syringes	65	14.1
	100	21.6
	30	5.8
Survey	65	12.5
	100	19.3

Although some of the assumptions made initially to compute the estimates have not been verified, the good agreement between the different indicators strengthens the obtained results. The findings support the previous assumption that injection is not the unique administration route of heroin. This is in agreement with findings from a study conducted in France (252), where the content of used syringes was analysed and the results showed that heroin was detected in only 49% of the samples, followed by cocaine (41%), buprenorphine (29%) and methylethylcathinone (21%). On the other hand, estimates derived from distributed syringes do not account for the proportion of heroin, which is consumed via fumigation and nasal insufflation. These were reported as the major administration routes by 18.4% and 26.3% of the respondents in the investigated area, respectively (171). Thus, one could infer that daily consumption of heroin via injection is likely closer to estimates obtained with 30 mg.dose⁻¹, i.e., 6.5 g.day⁻¹ of pure heroin. By subtracting these from wastewater-based estimates (12.7 g.day⁻¹), one could infer that the remaining 6.2 g.day⁻¹ correspond to heroin, which is consumed by fumigation and nasal insufflation. Interestingly, estimates derived from prevalence data (general population survey) using 65 mg.dose⁻¹ are in agreement with wastewater-based estimates. When confronted to syringe data, this would indicate that the average dose (for injection, fumigation and nasal insufflation) lies between 30 and 65 milligrams. This is in agreement with the supposedly lower bioavailability of heroin after fumigation or nasal insufflation (178), resulting in a need for a higher dose, compared to

intravenous injection. Based on these findings, one could hypothesize that the dosage and the consumption frequency considered as well as the number of users (i.e., 193) are an informative representation of the actual situation in the investigated area. By joining together all the available estimates, one could infer that the average daily consumption of heroin in the investigated area is approximately 13 grams per day (range 5.8 – 21.6), corresponding to 118 grams at 11% average street level purity. Although precisely estimating heroin consumption with these indicators remains a difficult task, mainly due to uncertainty of prescription/sell-in data and the important fluctuations observed, the consensus obtained with other sources of data allows to strengthen the obtained findings.

12.4 Conclusion

Using a detailed database about opioid substitution treatment, the expected amounts of methadone consumed in the investigated catchment were computed. These estimates were confronted to back-calculations made from methadone's metabolite (EDDP) and the obtained results were consistent with expectations. The findings illustrate an example in which wastewater data corresponds to data of other sources of information about consumption of a controlled substance. Furthermore, they allow assessing the reliability of the adopted sampling strategy, suggesting its ability to capture the consumption of a small cohort and could thus be used to monitor other substances. Concerning 6-monoacetylmorphine, further research is required before it can be used to estimate heroin consumption. Nonetheless, because it is not influenced by changes in prescription/sales of pharmaceuticals, it is likely a more suitable marker for longitudinal monitoring of heroin consumption compared to morphine. Although providing precise estimates of heroin consumption using the latter compound can prove difficult due to the uncertainty surrounding prescription/sales data, results obtained in the context of this study are in agreement with estimates obtained from other sources of data about heroin consumption. This allows to strengthen the information provided by the different indicators and to formulate hypothesis about the extent of heroin consumption in the investigated area. The present study illustrates an example in which a more balanced image of substance abuse can be obtained by the combination of wastewater analyses and epidemiological data. If further promoted, the combination of various and complementary indicators would help advancing the understanding and monitoring of substance abuse.

12.5 Acknowledgements

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12.6 Supporting Information

12.6.1 Wastewater sampling and processing

An autosampler (6712FR ISCO refrigerated (4°C) autosampler, Teledyne ISCO, Lincoln, NE) was used to collect wastewater samples (sampling frequency: 5 minutes, volume: 65 mL).

Prior to collection, sodium metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$, concentration: 2 g.L^{-1}) was added to the empty bottles used in the autosampler to reduce degradation of the analytes until collection (112). Hourly samples were then mixed in a volume proportional manner to obtain a 24-hour composite sample (final volume: 150 mL). Daily samples were then filtered ($0.7 \mu\text{m}$ nitrocellulose filters, type GF/F Whatman, GE Healthcare), acidified using hydrochloric acid (37%, Sigma-Aldrich, St. Louis, MO, USA), spiked with deuterated standards of the target compounds and extracted using the procedure described by Been et al. (76). 0.5 mL of wastewater extract was evaporated to dryness under a gentle stream of nitrogen and reconstituted in 1 mL of water, with 0.1% of formic acid, and methanol (90:10, v/v).

12.6.2 Chemicals

Standards of methadone (MET), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), 6-acetylmorphine (6-MAM) and morphine (MOR) were purchased as 1 mg.mL^{-1} calibrated solutions (Lipomed AG, Arlesheim, Switzerland). Deuterated standards of methadone-d3, EDDP-d3, 6-MAM-d3 and MOR-d3 were purchased as 0.1 mg.mL^{-1} calibrated solutions (Lipomed AG, Arlesheim, Switzerland). Hydrochloric acid (37%) and ammonium hydroxide ($\geq 25\%$) were TraceSelect® grade (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). Water, methanol and acetonitrile were Ultra Chromasolv® grade (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland).

12.6.3 Instrumental analysis

Analyses were performed on 1290 Infinity UHPLC (Agilent, Santa Clara, CA, USA) coupled to a 5500 QTrap triple quadrupole mass spectrometer (ABSciex, Ontario, Canada) interfaced with an electrospray ionization (ESI) operated in positive-ion mode. Analytes were separated on a Kinetex Core-Shell C18 (Phenomenex, Torrance, CA, USA) column (particle size $2.6 \mu\text{m}$, $100 \times 2.1 \text{ mm}$) at a constant flow rate of 1.2 mL/min . The autosampler was kept at 4°C and the column at 40°C . Mobile phases consisted in water with 0.1% of formic acid (solvent A) and acetonitrile (solvent B). Proportion of solvent B was increased from 5 to 10% at 1.8min and then increased linearly to 100% at 7min, at 7.2min the proportion was decreased to 5% until 8.5min. Air and nitrogen were used as nebulizer and collision gas, respectively. Desolvation temperature was set to 600°C and the ion source was set to 5.5 kV. The mass spectrometer (MS/MS) was operated in scheduled multiple reaction monitoring (sMRM). Details about the method are reported in Table 29.

Table 29: Summary of MS/MS parameters. Rt: Retention time, DP: declustering potential, CE: collision energy.

	<i>R_t</i>	<i>Internal Standard</i>	<i>Parent [m/z]</i>	<i>Fragment [m/z]</i>	<i>DP (Volt)</i>	<i>CE (Volt)</i>
MOR	0.32	MOR-d3	286.1	152.1	55	74
				128	55	76
6-MAM	1.27	6-MAM-d3	328.1	211.2	60	35
				165.1	60	51
EDDP	3.39	EDDP-d3	278.2	186.1	60	45
				219.1	60	58
MET	3.64	MET-d3	310.1	265.2	60	20
				105	60	34

Calibration was carried out using the previously described standards in the concentration range from 10 to 1500 ng/L (internal standards were added at the concentration of 250 ng/L). Calibration was conducted prior to each analytical sequence. Accuracy and precision (206) were determined using blank drinking water spiked with the target compounds at three different concentration levels (50, 250 and 1250 ng.L⁻¹). Analyses were conducted for three consecutive days. The method quantification limit (MQL) was calculated using blank drinking water samples, spiked with target compounds at decreasing concentration, and extracted using the abovementioned procedure. The MQL was determined as the lowest analyte concentration giving a signal-to-noise ratio greater than 10 (206). Extraction recoveries were determined by spiking wastewater samples (n=3) with deuterated standards before (pre-extraction aliquot) and after (post-extraction aliquot) solid-phase extraction, as suggested by (207,208)). The recovery was calculated as the ratio between the signal of the deuterated standards in the pre- and post-extraction aliquots.

12.6.4 Flow measurements – Regression

Daily flow data consisted in measurements (m³.s⁻¹) at 5 minutes intervals, for a total of 288 points per day. Total daily flows were calculated as the sum of the individual intervals. To estimate the corresponding standard error, a third order Gaussian peak fitting model was used to fit the data, as shown in Equation 1.

$$y = \sum_{i=1}^n a_i e^{\left[-\left(\frac{x-b_i}{c_i}\right)^2\right]} \quad \text{Equation 1}$$

where *a* is the peak amplitude, *b* is the centroid, *c* is a factor related to the peak's width and *n* is the number of peaks (in this case *n* = 3). The sum of squares of the residuals from the regression were used to estimate the standard error (SE) associated to each measurement, as shown in Equation 2,

$$SE = \sqrt{\frac{\sum_{i=1}^n \varepsilon_i^2}{n-4}} \quad \text{Equation 2}$$

where ε_i is the residual from the i^{th} flow measurement interval and n is the number of degrees of freedom ($n = 288$).

12.6.5 Syringes distribution – Historic data

Data from a survey conducted in 2011 (245) in 220 out of 248 pharmacies was used to estimate the proportion of syringes supplied by the latter versus the total number of syringes distributed in the catchment (low threshold facilities + pharmacies). During the period considered by the survey (October to December 2010), 9895 ± 322 were distributed on average by low threshold facilities, while an estimated 3482 syringes were distributed by the pharmacies in the investigated area, corresponding to 26% of the total (245). By assuming that changes in the number of distributed syringes, which might have occurred since the survey was conducted, affected similarly both low threshold facilities and pharmacies, this proportion was used on the contemporary figures to estimate the total number of distributed syringes.

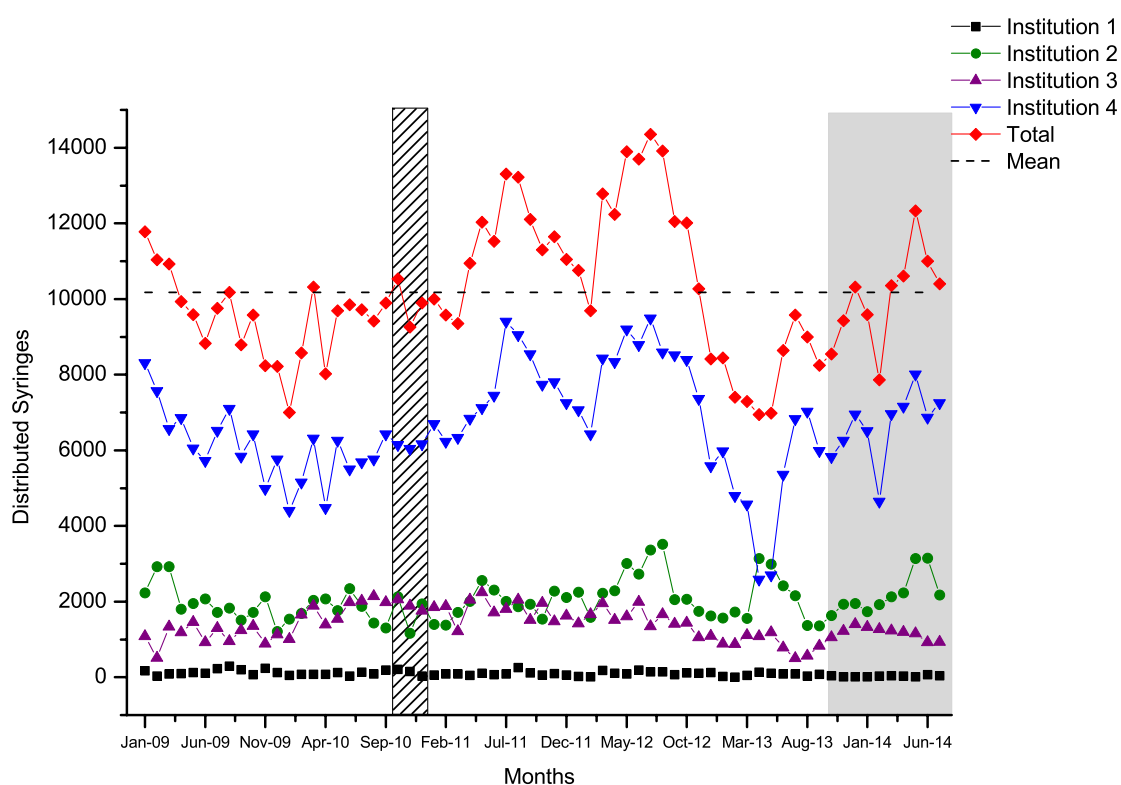


Figure 40 Syringe distribution by the four low threshold facilities in the investigated area. The striped area marks the three months period during which the proportion of syringes supplied by pharmacies was investigated and measured. The grey area marks the period investigated in the context of this study (October 2013 – June 2014).

12.6.6 Heroin prevalence data

According to the latest figures, yearly heroin prevalence in Switzerland was estimated to 0.1% of the population aged 15 to 75 and more (data from 2012) (253). Extrapolating to the catchment, this age group corresponds 85.3% of the total population (i.e., 226'000) (254), thus there would be approximately 193 individuals reporting consumption at least once in 2012. If

one assumes that the size of the cohort has not changed, that each individual consumes on one dose per day the daily consumption would be between 5.8 and 19.3 g/day of pure heroin, as shown in Table 30.

Table 30: Estimated heroin consumption based on prevalence data and three different dosages.

	<i>Dosage</i>			<i>Mean (SD)</i>
	30 mg	65 mg	100 mg	
<i>Estimated heroin consumption [g/day]</i>	5.8	12.5	19.3	12.5 (6.7)

12.6.7 Wastewater analysis – Methadone

Table 31: Methadone and EDDP loads estimates based on back-calculations with MET and EDDP. Results are reported with and without considering adsorption onto particulate matter.

	<i>Methadone [g.day⁻¹]</i>			
	<i>L_{EDDP,WW} (n = 24)</i>		<i>L_{Methadone,WW} (n = 26)</i>	
	<i>Without adsorption</i>	<i>With adsorption</i>	<i>Without adsorption</i>	<i>With adsorption</i>
<i>Mean</i>	75.6	99.4	41.7	47.5
<i>SD</i>	19.3	26.7	8.7	9.9

12.6.8 Wastewater analysis – Heroin and morphine

Table 32: Amount of pure heroin consumed in the catchment estimated from 6-MAM and morphine ($L_{6\text{-MAM,WW}}$, $L_{\text{Morphine,WW}}$) loads using Monte Carlo simulations after exclusion of outliers.

	<i>Heroin [g.day⁻¹]</i>		
	<i>L_{6-MAM,WW} (n=24)</i>	<i>L_{Morphine,WW} (n=26)</i>	
	<i>Without adsorption</i>	<i>Without adsorption</i>	<i>With adsorption</i>
<i>Mean</i>	542.1	51.6	53.8
<i>SD</i>	163.4	8.9	9.3

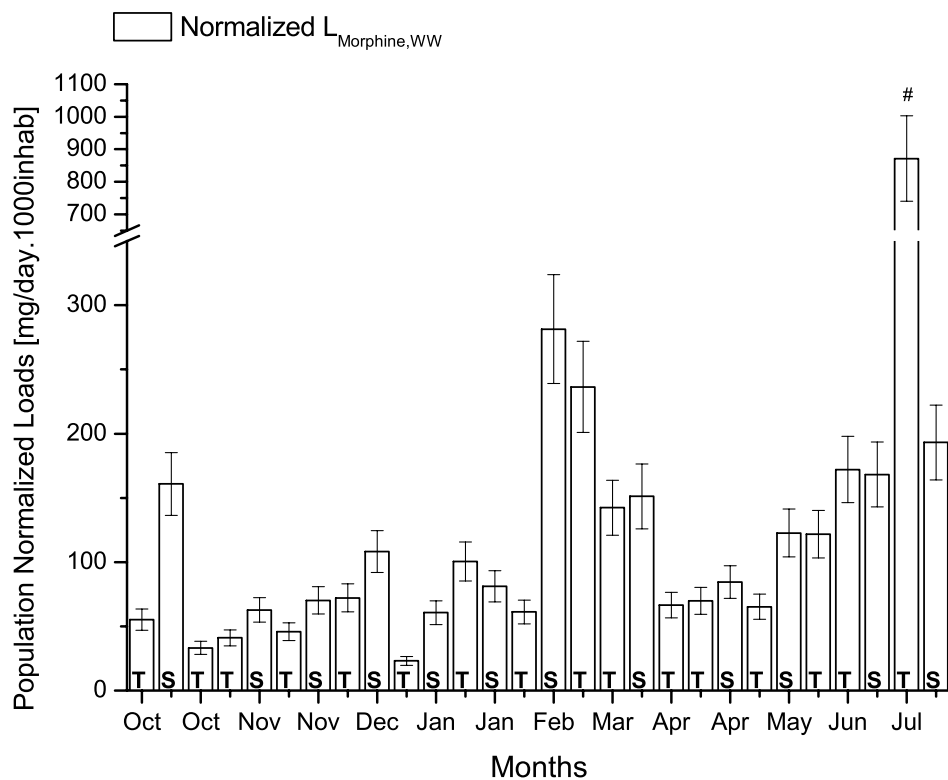


Figure 41: Population normalized morphine loads (milligrams per day per 1000 inhabitants) as described by Been et al. (76). (#) Outlier.

XIII Annexe III: Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland

Been F., Bijlsma L., Benaglia L., Berset J-D., Botero-Coy A. M., Castiglioni S., Kraus L., Zobel L., Schaub M., Bücheli A., Hernández F., Delémont O., Esseiva P., Ort C.; *Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland.* **submitted.**

13.1 Abstract

Background: Wastewater analysis is an innovative approach that allows monitoring illicit drug use at the community level. This study focused on investigating geographical differences in drug consumption by comparing epidemiological, crime and wastewater data. **Methods:** Wastewater samples were collected in 19 cities across Germany and Switzerland during one week, covering a population of approximately 8.1 million people. Self-report data and consumption offences for the investigated areas were used for comparison and to investigate differences between the indicators. **Results:** Good agreement between data sources was observed for cannabis and amphetamine-type stimulants, whereas substantial discrepancies were observed for cocaine. In Germany, an important distinction could be made between Berlin, Dortmund and Munich, where cocaine and particularly amphetamine were more prevalent, and Dresden, where methamphetamine consumption was clearly predominant. Cocaine consumption was relatively homogenous in the larger urban areas of Switzerland, although prevalence and offences data suggested a more heterogeneous picture. Conversely, marked regional differences in amphetamine and methamphetamine consumption could be highlighted. **Conclusions:** Combining the available data allowed for a better understanding of the geographical differences regarding prevalence, typology and amounts of substances consumed. For cannabis and amphetamine-type stimulants, the complementarity of survey, police and wastewater data could be highlighted, although notable differences could be identified when considering more stigmatised drugs (*i.e.*, cocaine and heroin). Understanding illicit drug consumption at the national scale remains a difficult task, yet this research illustrates the added value of combining complementary data sources to obtain a more comprehensive and accurate picture of the situation.

Keywords: Illicit drugs, epidemiology, surveys, crime statistics, wastewater

13.2 Introduction

The consumption of illicit drugs in a given population or community remains a partially hidden activity that cannot be directly measured nor totally unveiled. Traditionally, the nature, versatility and extent of this phenomenon are assessed through the use of indicators such as consumption surveys or descriptive statistics. The last decade has seen the emergence and refinement of wastewater-based epidemiology (WBE), which relies on quantitative measurement of specific biomarkers of illicit drug use in wastewater. Presented as a complementary approach to current surveillance methods (242,243,255–257), WBE has received much attention. Although further developments will most probably refine it in the near future, WBE already enables the gathering of unique spatio-temporal information about consumption (136). Nevertheless, information about consumers (e.g., age, sex, socio-economical status and history of drug use), crucial for policy makers, cannot be gathered by this approach, but requires the use of classical monitoring tools. Yet, these are also affected by some limitations such as the difficulty to obtain representative estimates, long study times, high costs and difficulties in reaching specific groups of regular users (136,179).

In general, estimating and monitoring drug use relies on direct and indirect methods (7). The former are mainly based on general population surveys (GPS), where a representative sample of the population is questioned about illicit drug use. The latter rely on extrapolating information about drug use from other sources indirectly related to drug use such as police statistics, treatment data as well as WBE. Despite suffering from the abovementioned limitations, these methods provide a partial, yet informative perspective of the phenomenon. By bringing together different and complementary data sources, it is expected to obtain a more precise understanding of the dynamics of illicit drug use at the national level.

In this study, samples collected from different wastewater treatment plants (WWTP) in Germany and Switzerland (including Liechtenstein) were analysed. Estimates of the average daily consumption of illicit drugs were computed based on these measurements and pharmacokinetics data available in the literature. Results obtained from wastewater analysis were compared to data derived from GPS and consumption offences registered by police forces. The aim of this study was to evaluate geographical differences and formulate hypotheses explaining divergences in the data sets. The cities investigated in the context of this study are shown in Figure 42. Focus was set on the use of cocaine, cannabis, heroin, amphetamine, methamphetamine, and 3,4-methylene-dioxymethamphetamine (MDMA or ecstasy).

13.3 Materials and methods

13.3.1 Epidemiological data and police statistics

General population survey data included the reported prevalence of use during the 12-months prior to questioning with a focus on the investigated areas (see Figure 42 and Table 33) (9,180–184). Reported substances were: cocaine, cannabis, amphetamine and

amphetamine-type stimulants (ATS) – such as methamphetamine and MDMA (for Germany only) and heroin (for Switzerland only). For Germany, available data was representative of the Federal States (Bundesland) (182–184), except for Berlin, where capture/recapture methods were used to derive the estimates (180,181). For Switzerland, survey data included responses provided by participants living within the catchments of the considered WWTP (9). See *Section 13.8.1* for more details about GPS data used.

Police statistics consisted of the number of offences for illicit drug use registered in the investigated areas during 2013 (Switzerland) and 2014 (Germany). Data were expressed as number of offences per year per thousand inhabitants. Epidemiological data and police statistics were not available for Liechtenstein.

13.3.2 Wastewater data

Amphetamine, methamphetamine and MDMA were quantified in wastewater samples together with the specific urinary metabolites of cocaine (*i.e.*, benzoylecgonine), cannabis (*i.e.*, 9-carboxy-delta-9-tetrahydrocannabinol, THC-COOH) and heroin (6-monoacetylmorphine, 6-MAM).

Daily 24-hour composite raw wastewater samples were collected over 7 consecutive days in March 2014, from 19 cities (in total 22 WWTPs) across Germany and Switzerland (including Liechtenstein), as shown in Figure 42 and Table 33.



Figure 42: Cities investigated in the context of the study.

Annexe III: Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland

Table 33: Summary of major characteristics of the sampled WWTP and period when the considered surveys were carried out. a) Reported population corresponds to figures provided by the WWTP personnel. b) No samples collected on weekend. TP: Time-proportional, VP: Volume-proportional (*2h time-prop. 24h volume-prop.), FP: Flow-proportional, NA: Not available. Grey shading: no previous WBE data (first WBE data in this study)

Country	City/WWTP	^a Population	Typical daily flow [m ³ .day ⁻¹]	Sampling Approach	Period	Survey
Germany	Dortmund	371'788	90'000	NA	12.03-18.03.14	2000
	Dülmen	34'495	7'500	TP	12.03-18.03.14	2000
	Dresden	593'050	110'000	VP	11.03-17.03.14	2009
	Munich	1'000'000	330'000	TP	12.03-18.03.14	2009
	Berlin Münchehofe	290'000	42'000	VP	11.03-14.03.14, 17.03.14	2010-2012
	Berlin Ruhleben	1'300'000	210'000	VP*	10.03-16.03.14	
	Berlin Schönerlinde	750'000	85'000	VP*	11.03-17.03.14	
	Berlin Wassmannsdorf	1'500'000	180'000	VP*	11.03-17.03.14	
Switzerland	Basel	260'000	77'000	VP	18.03-24-03.14	2013-2014
	Bern	206'655	65'000	VP	18.03-24-03.14	2013-2014
	Biel	82'285	37'065.6	VP	18.03-24-03.14	2013-2014
	Chur	52'800	12'000	FP	18.03-24-03.14	2013-2014
	Geneva	417'200	140'000	VP	18.03-24-03.14	2013-2014
	Lausanne	220'000	108'000	FP	18.03-24-03.14	2013-2014
	Lugano	103'000	50'000	TP	18.03-24-03.14	2013-2014
	Lucerne	174'800	75'000	VP	18.03-24-03.14	2013-2014
	Neuchatel	50'000	17'000	VP	18.03-24-03.14	2013-2014
	Sion	45'000	20'380	TP	18.03-24-03.14	2013-2014
	St.Gallen	52'000	17'800	VP	18.03-24-03.14	2013-2014
	Winterthur	125'000	40'000	VP	18.03-24-03.14	2013-2014
	Zurich	410'000	170'000	VP	18.03-24-03.14	2013-2014
Liechtenstein	Bendern	74'000	25'000	TP	18.03-24-03.14	NA

Wastewater samples were analysed using validated liquid-chromatographic tandem mass spectrometric methods. Details on the analytical procedures can be found in (130) (samples from Germany), (86) (Lugano), (256) (Chur, Lausanne, Lucerne, Neuchatel and Sion) and (57) (Basel, Bern, Geneva, St. Gallen, Zurich, Winterthur and Liechtenstein (Bendern)). All laboratories are involved in the multi-city study published by the EMCDDA (258) and the analytical performance of methods used were assured by the participation in external inter-laboratory exercises.

Average daily population normalised loads (concentrations multiplied by daily wastewater flows and divided by the number of inhabitants), back-calculations (daily consumption of parent compound based on estimated loads and excretion data) and the associated errors were estimated for each city using Monte Carlo simulations, following existing procedures (147,256). The parameters used are reported in Table 34 Table 35. For Berlin, the results from the four WWTPs were merged to be representative of the catchment area covering the entire city.

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Table 34: General parameters used in Monte Carlo simulations to estimate daily mass loads [g.day⁻¹] of illicit drugs and their metabolites. μ = mean, SE = standard error.

	μ	SE	Distribution	Note
Flow	Daily flow [L.day ⁻¹]	20% of daily flow	Normal (μ , SE ²)	Based on findings by Ort et al. (95,100), the error associated to daily flow measurements (S.E.) was estimated as 20% of the total daily flow
Substance concentration	Measured concentration [ng.L ⁻¹]	25% of measured concentration	Normal (μ , SE ²)	From a preliminary evaluation of an inter-laboratory test, an average deviation of 25% from the expected values was reported by the participating laboratories.

Table 35: Compound specific parameters used in Monte Carlo simulations to back-calculate the amounts of parent compound initially consumed. μ = mean, SE = standard error.

	Substance	μ	SE	Distribution	Note
Excretion rates	Benzoyllecgonine	30.58%	3.35%	Beta (a,b)	Inverse-variance weighted average. Data derived from summary in (35,186)
	MDMA	15.78%	1.83%		
	Amphetamine	29.12%	0.93%		
	Methamphetamine	28.56%	2.59%		

Nationwide estimates of illicit drug use were computed using a series of ordinary least squares and mixed effect models. The back-calculated amount of parent compound consumed in each city per day of the week was used as response variable. The number of inhabitants was used as predictor variable in ordinary regression analysis, while for mixed effect models, the day of the week was also included as (random) predictor variable. Additional information about wastewater data can be found in *Section 13.8.2*.

13.4 Results

13.4.1 Population Surveys

Estimates of last-year prevalence of cocaine, cannabis and ATS in the investigated cities are shown in Figure 43-Figure 46 and Table 36.

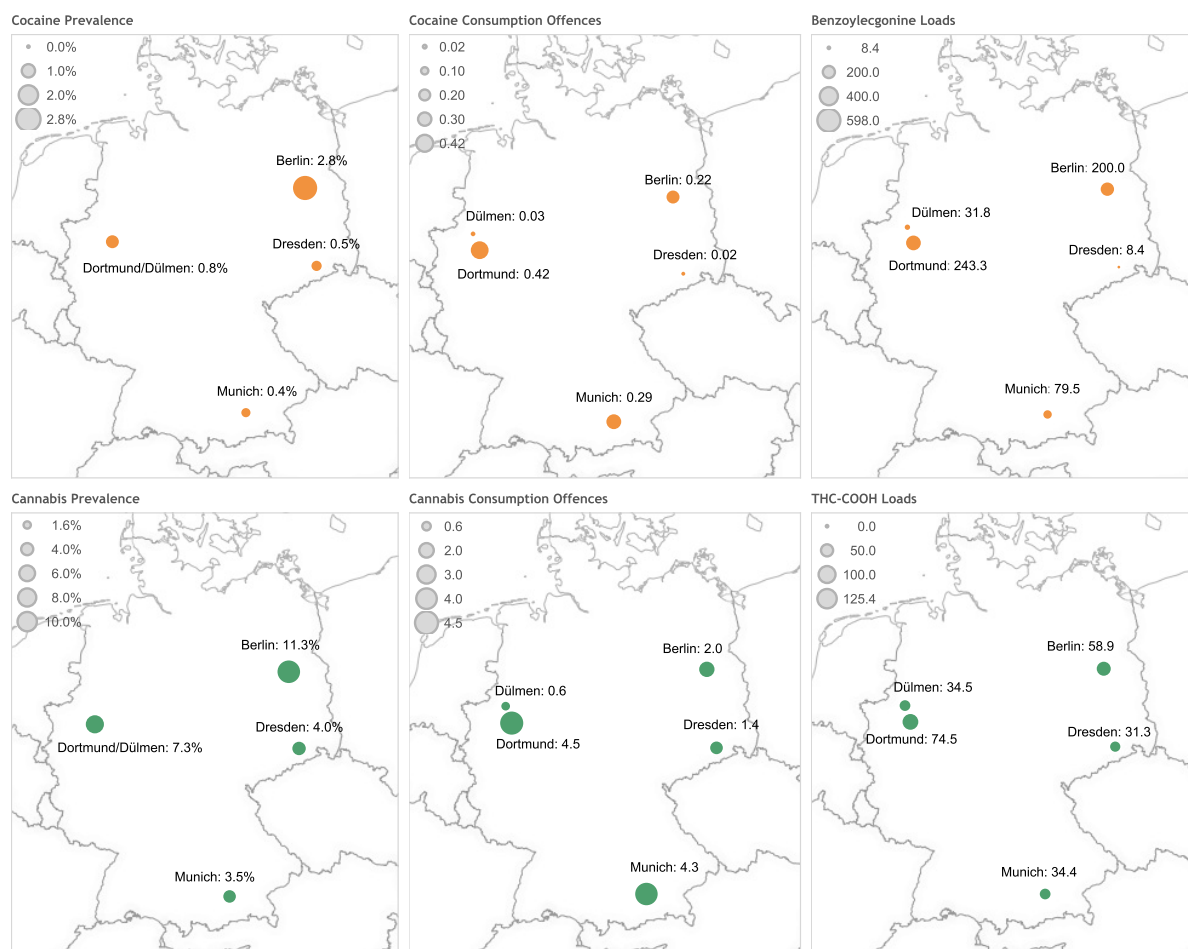


Figure 43: 12-months prevalence for cocaine and cannabis in Berlin and the federal states of Northrhine-Westfalia (Dortmund and Dülmen), Saxony (Dresden) and Bavaria (Munich). Reported consumption offences per year per thousand inhabitants. Average population normalised loads [mg.day⁻¹.1000inhab⁻¹]. Only data from three of the four WWTP sampled in Berlin are reported (weekend data were missing for Berlin-Münchehofe).

Annexe III: Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland

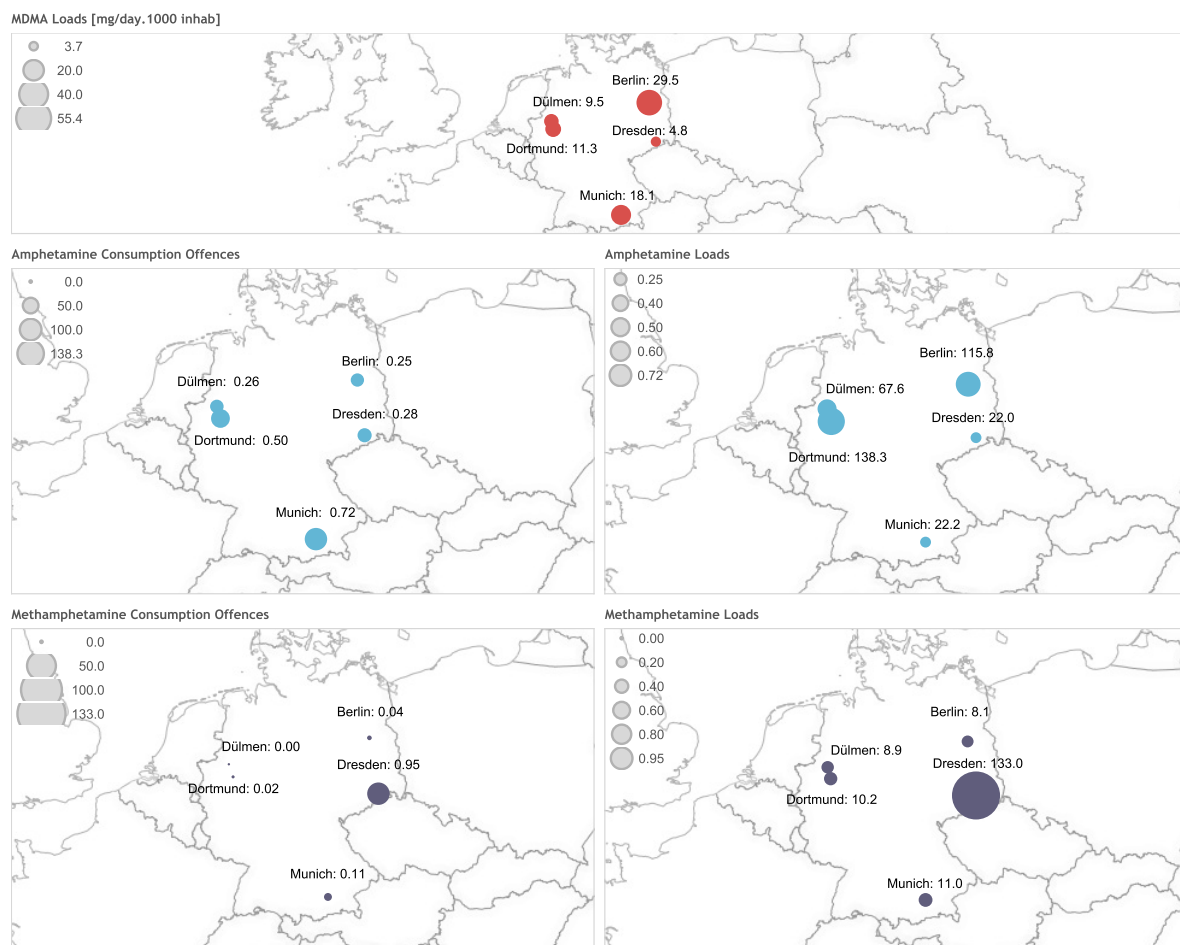


Figure 44: Average population normalised loads [mg · day⁻¹ · 1000 inhabitants⁻¹] for MDMA, amphetamine and methamphetamine measured in Germany. Reported offences per year per thousand inhabitants in Germany. Offences data for MDMA was not available.

Annexe III: Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland

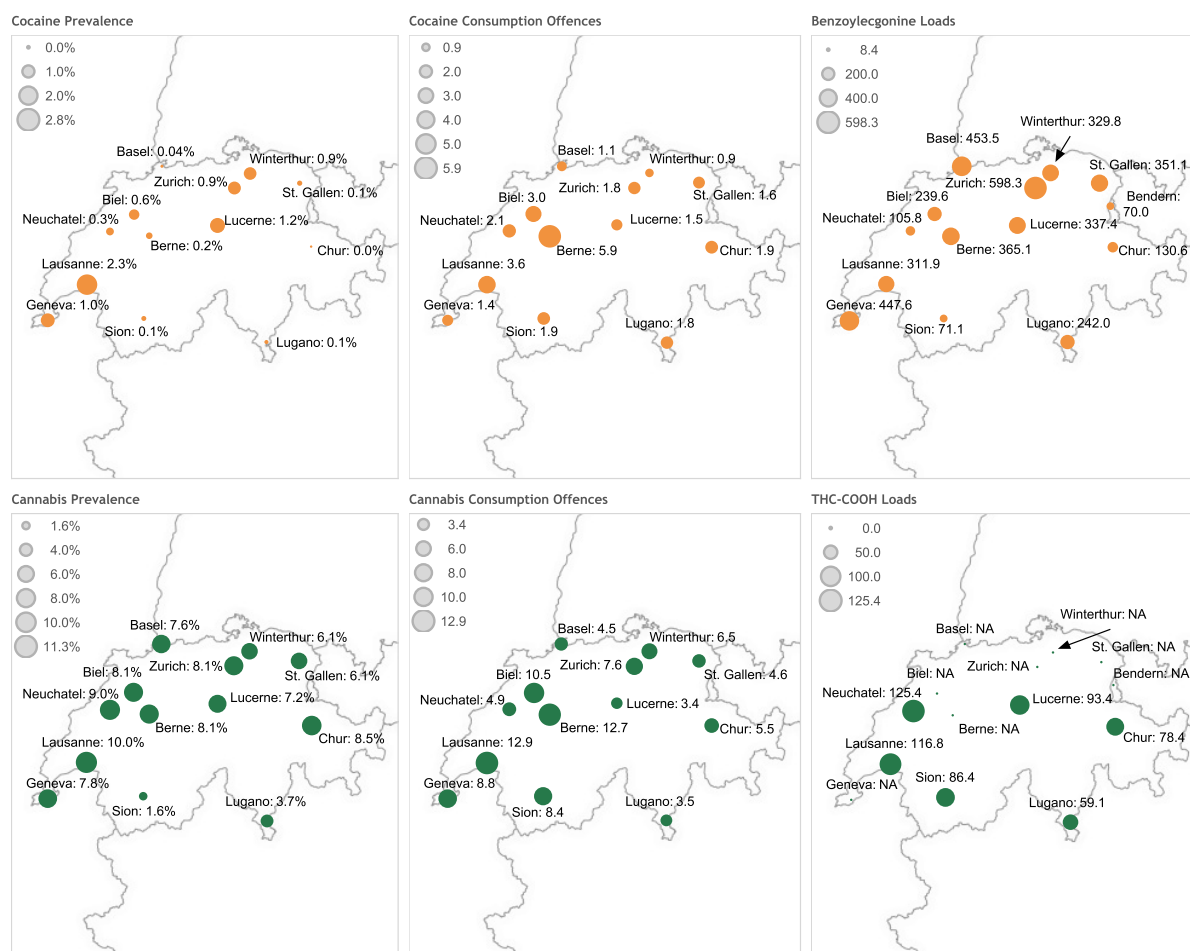


Figure 45: 12-months prevalence, reported consumption offences per year per thousand inhabitants and average population normalized loads [mg.day⁻¹.1000 inhabitants⁻¹], for cocaine and cannabis in Switzerland and Lichtenstein (wastewater data only). Wastewater samples from Geneva, Bern, Biel, Basel, Winterthur, Zurich and Bendern, were not analysed for THC-COOH.

Annexe III: Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland

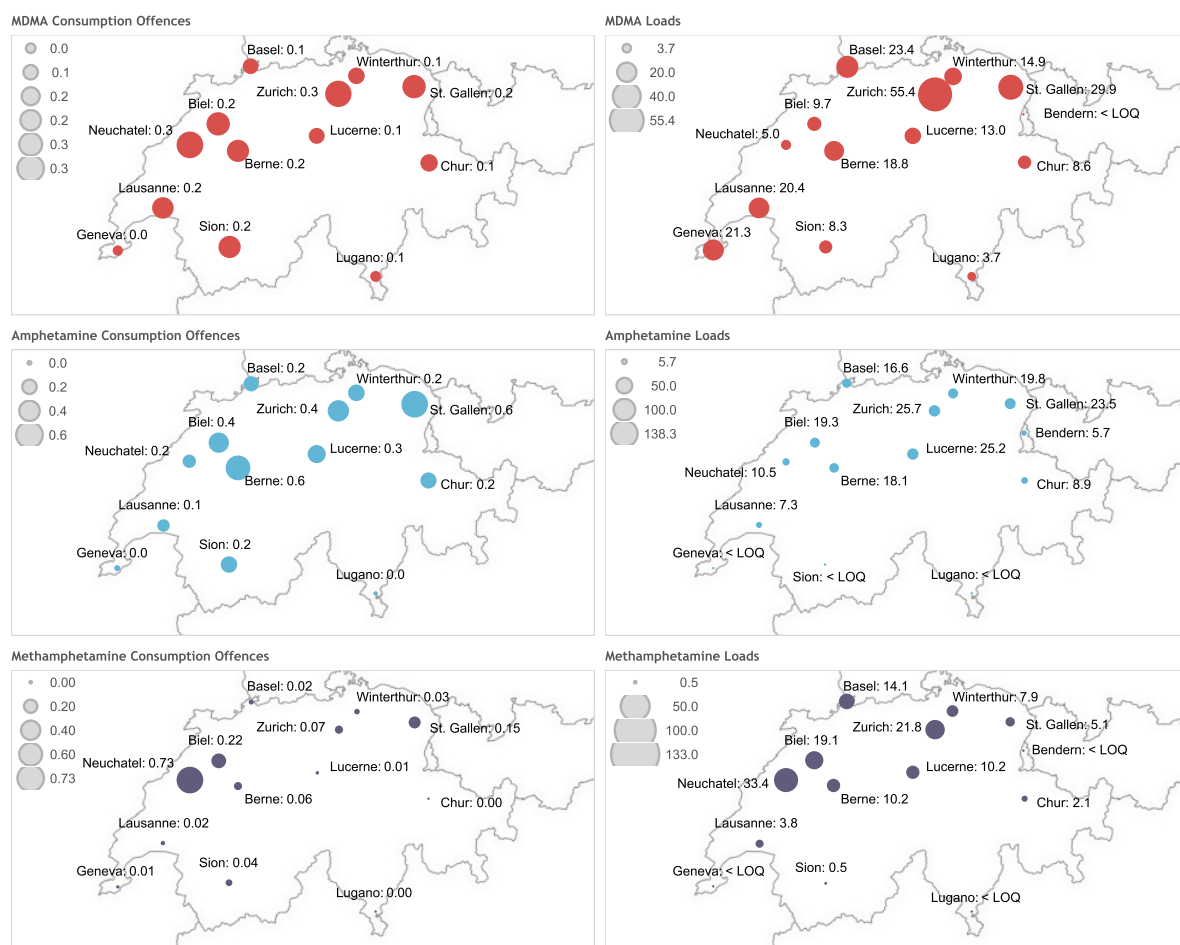


Figure 46: Average population normalised loads [mg.day⁻¹.1000 inhabitants⁻¹] for MDMA, amphetamine and methamphetamine measured in Germany. Reported offences per year per thousand inhabitants in Germany. Offences data for MDMA was not available.

Annexe III: Assessing geographical differences in illicit drug consumption – A comparison of results from epidemiological and wastewater data in Germany and Switzerland

Table 36: Summary of prevalence, offences (per thousand inhabitants) and wastewater (in [mg.day⁻¹.1000 inhabitants⁻¹]) data for all cities investigated in the context of this study. a) Population normalised loads of benzoylecgonine, the major metabolite of cocaine; b) Population normalised loads of 6-monoacetylmorphine, the exclusive metabolite of heroin.

City	Cocaine				Cannabis			MDMA	
	Prevalence	Offences	^a Wastewater	Survey	Offences	Wastewater	Survey	Offences	Wastewater
Basel	0.04%	1.13	453.5	7.6%	4.52	-	-	0.1	23.4
Berne	0.2%	5.93	365.1	8.1%	12.68	-	-	0.2	18.8
Biel	0.6%	3.01	239.6	8.1%	10.53	-	-	0.2	9.7
Chur	0%	1.91	130.6	8.5%	5.46	78.4	-	0.1	8.6
Geneva	1.0%	1.42	447.6	7.8%	8.77	-	-	0	21.3
Lausanne	2.3%	3.58	311.9	10.0%	12.95	116.8	-	0.2	20.4
Lugano	0.1%	1.81	242.0	3.7%	3.54	59.1	-	0.1	3.7
Lucerne	1.2%	1.46	337.4	7.2%	3.37	93.4	-	0.1	13.0
Neuchatel	0.3%	2.06	105.8	9.0%	4.86	125.4	-	0.3	5.0
Sion	0.1%	1.87	71.1	1.6%	8.44	86.4	-	0.2	8.3
St. Gallen	0.1%	1.64	351.1	6.1%	4.64	-	-	0.2	29.9
Winterthur	0.9%	0.85	329.8	6.1%	6.51	-	-	0.1	14.9
Zurich	0.9%	1.79	598.3	8.1%	7.60	-	-	0.3	55.4
Bendern	-	-	70.0	-	-	-	-	-	< LOQ
Dortmund	0.8%	0.42	243.3	7.3%	4.53	74.5	-	-	11.3
Dülmen	-	0.03	31.8	-	0.64	34.5	-	-	9.5
Dresden	0.5%	0.02	8.4	4.0%	1.36	31.3	-	-	4.8
Munich	0.4%	0.29	79.5	3.5%	4.28	34.4	-	-	18.1
Berlin	2.8%	0.22	200.0	11.3%	2.00	58.9	-	-	29.5

Table 36 (continued)

City	Amphetamine		Methamphetamine		Heroin	
	Offences	Wastewater	Offences	Wastewater	Offences	^b Wastewater
Basel	0.21	16.6	0.02	14.1	0.63	7.4
Berne	0.55	18.1	0.06	10.2	5.65	7.9
Biel	0.37	19.3	0.22	19.1	3.57	8.8
Chur	0.24	8.9	0	2.1	1.14	< LOQ
Geneva	0.03	< LOQ	0.01	< LOQ	1.73	9.6
Lausanne	0.14	7.3	0.02	3.8	3.57	7.2
Lugano	0.02	< LOQ	0	< LOQ	0.41	6.7
Lucerne	0.30	25.2	0.01	10.2	0.82	7.1
Neuchatel	0.16	10.5	0.73	33.4	0.67	< LOQ
Sion	0.25	< LOQ	0.04	0.5	0.62	1.9
St. Gallen	0.64	23.5	0.15	5.1	3.03	8.8
Winterthur	0.25	19.8	0.03	7.9	0.86	13.4
Zurich	0.41	25.7	0.07	21.8	0.64	17.7
Bendern	-	5.7	-	< LOQ	-	< LOQ
Dortmund	0.50	138.3	0.02	10.2	-	-
Dülmen	0.26	67.6	0.00	8.9	-	-
Dresden	0.28	22.0	0.95	133.0	-	-
Munich	0.72	22.2	0.11	11.0	-	-
Berlin	0.25	115.8	0.04	8.1	-	-

The highest prevalence for cocaine (2.8%) was measured for Berlin, with much lower levels in the other Federal States (0.4-0.8%). In Switzerland, Lausanne was highest (2.3%), followed by Lucerne (1.2), Geneva (1.0%), Zurich (0.9%) and Winterthur (0.9%). For cannabis, the highest prevalence in Germany were reported for Berlin (11%) and Dortmund/Dülmen (7.3%), with lower figures for Dresden (4.0%) and Munich (3.5%). In Switzerland, prevalence of cannabis use was far more homogeneous (average $7.0 \pm 2.2\%$), except for Sion and Lugano with very low levels (1.6% and 3.7%, respectively). Last-year prevalence data for amphetamine and ATS was available only for Germany, with the highest figures reported for Berlin and Dresden (see Figure 48).

13.4.2 Police statistics

Reported consumption offences per thousand inhabitants in German and Swiss cities are illustrated in Figure 43-Figure 46 and Table 36. Highest rates for cocaine in Germany were reported in Dortmund (0.42), followed by Munich (0.29) and Berlin (0.22). Particularly low offence rates were reported in Dresden and Dülmen. In Switzerland, highest rates were reported in Bern (5.9), Lausanne (3.6) and Biel (3.0). Heroin-related data was only available for Switzerland, where it depicted a situation similar to cocaine (see Figure 49). For cannabis, highest rates were reported in Munich and Dortmund (4.3 and 4.5, respectively), with the remaining cities ranging between 0.6 and 2. Slightly higher cannabis-related offences were reported in western Switzerland (between 8.4 and 13), compared to the eastern and southern parts (between 3.4 and 7.6). Data about MDMA-related offences was available only for Switzerland, where it showed a heterogeneous situation. Amphetamine offences were quite homogeneous in the northern cities of Germany (range 0.25 to 0.5), while the highest rate was reported in Munich (0.72). In Switzerland, there was a clear difference between south/west and north/east, with the former reporting lower rates (0-0.25) compared to the latter (0.2-0.65). For methamphetamine, the number of offences was generally low in the German cities (0 to 0.11), except for Dresden which reported offence rates of 0.95. In Switzerland, low rates were reported (0-0.06), except for Neuchatel, Biel and St. Gallen, where these were substantially higher (0.15-0.73).

13.4.3 Wastewater data

13.4.3.1 Drug loads

Population normalised loads of target substances measured in WWTP in Germany, Switzerland and Liechtenstein are reported in Figure 43-Figure 46 and Table 36. Fluctuations in drug loads observed during the week are illustrated in Figure 50-Figure 51. Weekend samples for the WWTP of Berlin Münchenhofe were not available, thus, the results refer only to the WWTP of Berlin Ruhleben, Schönerlinde and Wassmannsdorf. The contribution of drug loads measured during the week in Münchenhofe was, however, limited (*i.e.*, only 3.3 % of the total), except for amphetamine which was in the same order of magnitude as the other WWTPs.

Among German cities, highest benzoylecgonine loads were measured in Dortmund and Berlin (243.3 and 200 mg.day⁻¹.1000inhab⁻¹). In Switzerland, highest per capita loads were measured in Zurich, followed by Basel and Geneva (598.2, 453.4 and 447.6 mg.day⁻¹.1000inhab⁻¹, respectively). Lowest loads were measured in BERN (LI) and Sion (approximately 70 mg.day⁻¹.1000inhab⁻¹).

For THC-COOH, a more homogeneous picture was obtained both in Germany and Switzerland. Nonetheless, Dortmund and Berlin still exhibited twice as high per-capita loads compared to the other German cities (74.5 and 58.9 versus approximately 30 mg.day⁻¹.1000inhab⁻¹). In Switzerland, loads ranged from 59 mg.day⁻¹.1000inhab⁻¹ in Lugano to 125 mg.day⁻¹.1000inhab⁻¹ in Neuchatel.

In Germany, highest MDMA loads were measured in Berlin, followed by Munich, Dortmund and Dülmen (29.5, 18.0, 10.7 and 9.5 mg.day⁻¹.1000inhab⁻¹ respectively). In Switzerland, loads ranged from 55.4 in Zurich to 3.7 mg.day⁻¹.1000inhab⁻¹ in Lugano. In BERN (LI), 3.6 mg.day⁻¹.1000inhab⁻¹ were measured.

Consumption of amphetamine seemed to be mainly localised in the cities of Berlin, Dortmund and Dülmen (ranging from 67.6 to 138.3 mg.day⁻¹.1000inhab⁻¹). In Switzerland, amphetamine was predominant in the north-eastern part of the country (8.9 to 25.6 mg.day⁻¹.1000inhab⁻¹), while its occurrence was less marked in western and southern parts of the country (0 to 10.5 mg.day⁻¹.1000inhab⁻¹). BERN (Liechtenstein) showed results similar to western Switzerland (5.7 mg.day⁻¹.1000inhab⁻¹).

Highest methamphetamine loads were measured in Dresden (133 mg.day⁻¹.1000inhab⁻¹). In Switzerland, consumption seemed to be mainly localized in the cities of Neuchatel, Zurich and Biel (33.4, 21.8 and 19.1 mg.day⁻¹.1000inhab⁻¹, respectively).

Occurrence of 6-MAM (exclusive metabolite of heroin) was monitored only in Switzerland, as shown in Figure 49. Highest loads were measured in the cities of Zurich, Winterthur and St. Gallen (17.7, 13.5 and 12.1 mg.day⁻¹.1000inhab⁻¹, respectively), while other large cities (> 100'000 inhabitants) were characterized by similar loads (between 6.7 and 10.9 mg.day⁻¹.1000inhab⁻¹). In smaller catchments, substantially lower per capita loads were measured (between 1.9 and 2.7 mg.day⁻¹.1000inhab⁻¹), except for Biel which was in the same range as larger urban areas. No 6-MAM was detected in BERN.

13.4.3.2 Consumption estimates

Using measured loads and excretion data reported in Table 35, the amounts of pure substance (*i.e.*, parent compound) initially consumed were back-calculated using Monte Carlo simulations. Results are reported in Table 37.

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Table 37: 7-day back-calculated average illicit drug consumption [grams.day⁻¹] (Standard Error). a) 7-day average calculated from three out four WWTP in Berlin (weekend included). b) 5-day average calculated from the four WWTP in Berlin (weekend excluded).

	Population	Cocaine (Benzoylcegonine)	MDMA	Amphetamine	Methamphetamine
Dortmund	371'788	313.2 (120.5)	26.8 (11.2)	176.9 (65.6)	13.4 (5)
Dülmen	34'495	3.8 (1.6)	2.1 (0.9)	8 (3)	1.1 (0.4)
Dresden	593'050	17.2 (6.6)	18.1 (7.9)	44.9 (17.1)	278.2 (104.1)
Munich	1'000'000	274.9 (115.4)	115.8 (51.3)	76.4 (30.3)	38.8 (14.4)
^a Berlin	3'550'000	2456.9 (597.1)	672.4 (172.2)	1413.8 (305.7)	102.1 (102.1)
^b Berlin	3'840'000	2212.1 (572.6)	690.1 (690.1)	1374.6 (310.7)	100.7 (26.4)
Basel	260'000	408.1 (155.8)	38.9 (17.3)	14.8 (5.8)	13 (4.8)
Bern	206'655	261.2 (98.7)	25 (12.1)	12.9 (4.7)	7.5 (2.9)
Biel	82'285	68.3 (26.2)	5.1 (2.3)	5.5 (2)	5.5 (2.3)
Chur	52'800	23.9 (9.3)	2.9 (1.3)	1.6 (0.6)	0.4 (0.2)
Geneva	417'200	646.4 (247.9)	56.9 (26.3)	6.8 (2.6)	4.5 (1.8)
Lausanne	220'000	237.5 (91.9)	28.8 (13.7)	5.5 (2.1)	3 (1.2)
Lugano	103'000	86.2 (32.8)	2.4 (1)	1.4 (0.5)	0.2 (0.1)
Lucerne	174'800	204.1 (81.5)	14.6 (6.9)	15.1 (5.7)	6.3 (2.4)
Neuchatel	50'000	18.3 (7.1)	1.6 (0.8)	1.8 (0.7)	5.9 (2.3)
Sion	45'000	11.1 (4.2)	2.4 (1.2)	0 (0)	0.1 (0.1)
St. Gallen	52'000	63.2 (24.4)	10 (4.4)	4.2 (1.8)	0.9 (0.4)
Winterthur	125'000	142.7 (55.6)	11.9 (5.2)	8.5 (3.1)	3.5 (1.4)
Zurich	410'000	849 (330.6)	145.7 (77.2)	36.2 (14.6)	31.5 (11.8)
Bendern (Li)	74'000	17.9 (6.9)	1.7 (0.7)	1.5 (0.6)	0.6 (0.2)

Nationwide consumption estimates are given only for Switzerland as the size of the population sampled during the campaign corresponds to approximately 27% of the total Swiss population (sampled population: 2.2 million, total population in 2013: 8.14 (259)). Furthermore, estimates were limited to cocaine and MDMA as these are the only two substances detected in all cities. Scatterplots of consumption estimates as a function of the population are illustrated in Figure 47.

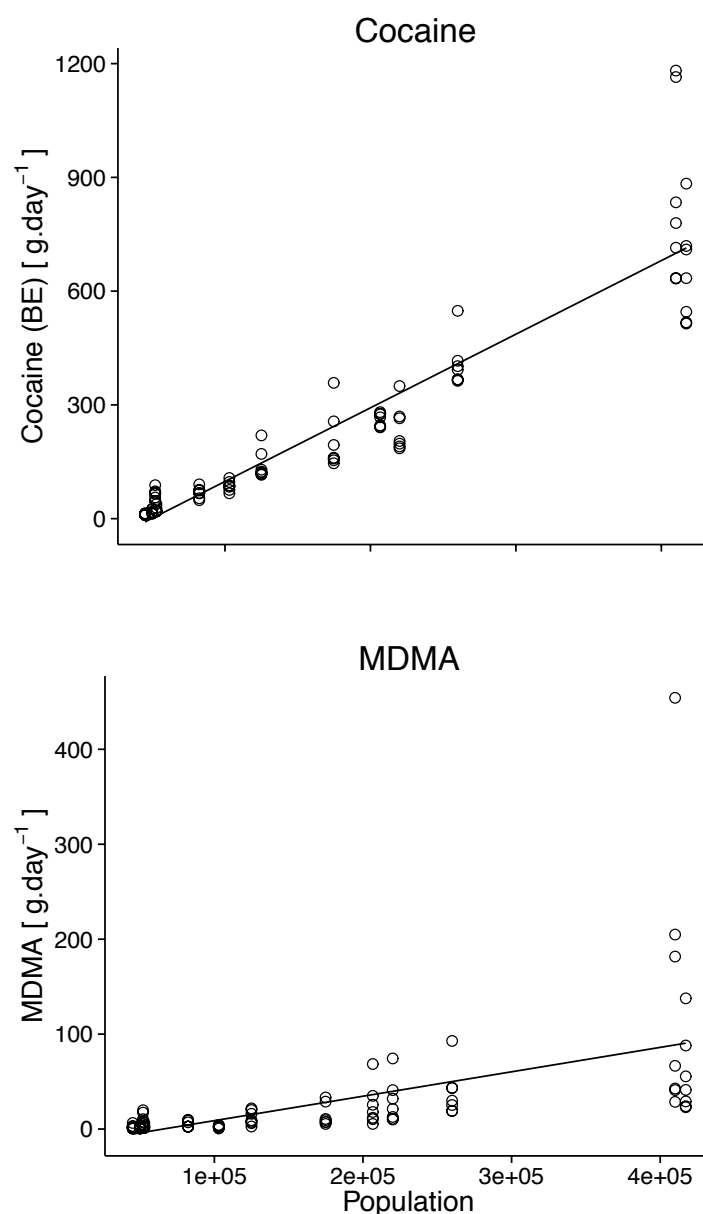


Figure 47: Scatter plot of estimated cocaine (based on benzoylecgonine loads) and MDMA consumption as a function of the size of the population. Line: ordinary least square.

Prior to regression analysis, data was log-transformed to correct for heteroscedasticity (*i.e.*, unequal variances between observations). For cocaine, a mixed effect model was used to extrapolate both, the daily average and weekly consumption estimate for municipalities not included in the wastewater sampling campaign (see *Section 13.8*, Figure 52 and Table 39 for further details). As reported in Table 41, the nationwide average daily consumption of cocaine was estimated to approximately 8.8 kg of pure substance. In terms of weekly consumption, 61.6 kg of pure cocaine was estimated.

For MDMA, mixed effect models did not provide satisfactory results, thus, an ordinary least squares model was used to extrapolate the nationwide daily average consumption which was estimated at 0.367 kg.day⁻¹ of pure MDMA (as reported in Table 38).

Table 38: Nationwide estimates for cocaine and MDMA [$\text{kg}\cdot\text{day}^{-1}$]. Total weekly figures were calculated as the sum of the values measured and extrapolated for each day of the week. For MDMA this was not possible and an ordinary least squares regression was used, which does not allow extrapolating day-by-day consumption.

		Cocaine (BE) [$\text{kg}\cdot\text{day}^{-1}$]	MDMA [$\text{kg}\cdot\text{day}^{-1}$]	Considered Population
Measured (Wastewater)	7-day average	3.0	0.346	2'198'740
	Weekly	21.2	2.4	
Extrapolated	7-day average	5.8	0.023	5'793'612
	Weekly	40.4	-	
Total	7-day average	8.8	0.367	7'992'352
	Weekly	61.6	-	

13.5 Discussion

13.5.1 Cocaine

In Germany, the overlap observed between prevalence, offences and wastewater data suggests that cocaine consumption is predominant in the cities of Dortmund and Berlin, although lower prevalence was reported in Dortmund. This difference, however, may be due to methodological differences between survey estimates in Dortmund (and other German cities) and capture/recapture estimates as applied in Berlin. Firstly, capture/recapture methods are less likely to produce underestimates, and secondly, the survey estimates used apply to regions (Bundesland) rather than to cities. For the city of Munich, offence data suggest a high occurrence of cocaine use, yet both prevalence and wastewater data indicate the contrary. In the case of Dresden, offence and wastewater data suggest limited cocaine consumption, whilst survey data indicate that prevalence is similar to Munich and Dortmund.

In Switzerland, conventional indicators depicted a highly heterogeneous situation, in particular between larger urban areas (*i.e.*, > 100'000 inhabitants), which showed substantial differences in prevalence and number of reported offences. Yet, these were less pronounced when observing results from wastewater analysis. Possible explanations for these discrepancies are reporting bias, concealment, stigmatization, city size, drug availability, as well as law enforcement activities, all of which strongly influence survey outcomes and offence rates. Still, an overlap between data sources was observed for urban areas with less than 100'000 inhabitants.

According to prevalence data, there is a slightly higher cocaine consumption in Swiss compared to German cities, which is confirmed by wastewater analysis. This is in contradiction with nationwide figures, which suggest that last-year prevalence is higher in Germany compared to Switzerland (*i.e.*, 0.8 and 0.5%, respectively (173,260)). Yet, the small number of cities analysed in Germany limits the interpretation.

Wastewater results obtained here were consistent with findings from the multi-city study reported by the EMCDDA (136,258).

Results from the analysis of benzoylecgonine were consistent with findings from the multi-city study reported by the EMCDDA (136,258).

13.5.2 Cannabis

Overall, prevalence and wastewater data showed a similar picture for cannabis consumption in Germany, while offence rates diverged slightly from the former indicators. Highest prevalence was reported for Berlin and Dortmund, with the latter having also the highest THC-COOH per capita loads. This could be due to its proximity to the Netherlands, where a more liberal drug policy on cannabis use is in force (135,261). Yet, no statistical difference in the measured loads could be found between Berlin and Dortmund (Wilcox Rank Sum p-value > α = 0.05). Similarly to cocaine, higher offence rates were reported in Munich, while both prevalence and wastewater data suggested that these were substantially lower than in Berlin and Dortmund.

Based on prevalence and wastewater data, cannabis consumption appear quite uniform across the investigated areas, except for the city of Sion where reported prevalence was particularly low compared to offence and wastewater results. In general, offence rates are more heterogeneous compared to the other two indicators. However, it should be noted that since October 1st 2013, use of cannabis by adults has been decriminalised and can now be punished with an administrative fine, without being recorded as a criminal offence (191).

Comparisons between the two countries suggest that cannabis consumption is slightly higher in Switzerland, which is in agreement with national prevalence figures (*i.e.*, 12-months prevalence of 4.5% and 5% in Germany and Switzerland, respectively (173,260)).

Results from wastewater analysis obtained in this context were consistent with findings from the multi-city study reported by the EMCDDA (136,258).

13.5.3 Amphetamine-type stimulants

The available indicators suggest that methamphetamine consumption in Germany was predominant in Dresden, while amphetamine consumption was substantially lower compared to the others cities. The occurrence of methamphetamine in Dresden is known to be related to its proximity to the Czech Republic, an important methamphetamine producer (90). Interestingly, available data suggests that consumption of methamphetamine is limited elsewhere, including Berlin and Munich, which are both in the eastern part of the country and thus, potentially affected by imports of methamphetamine from the Czech Republic. However, this is in agreement with the supposedly small-scale production (*i.e.*, kitchen laboratories), whose outputs are thought to supply predominantly domestic and neighbouring markets (262). Despite recent signs that its consumption is spreading (2), our data suggest that ATS consumption is still limited to border areas.

The proximity of Dortmund to the Netherlands, which is known for its ATS production (2,262), does not seem to have an effect on the availability and consumption of these substances. In

fact, amphetamine loads were in the same range as those measured in Berlin and MDMA loads were below the values measured in Munich. Whilst comparisons with prevalence data should be interpreted carefully, as these do not distinguish between the type of ATS reported, our results confirm the more widespread use of amphetamine compared to methamphetamine (2,262). Similar findings were obtained in a recent study where wastewater samples from various cities in western Germany were analysed (263).

Catchment specific prevalence data on ATS was not available for Switzerland, yet offence rates and wastewater data for both MDMA and amphetamine showed similar patterns. The occurrence of MDMA seems to be predominant in larger urban areas, while smaller cities are generally less affected. For amphetamine, both data sources suggest higher prevalence rates in the north-eastern part of Switzerland. Offences related to methamphetamine use were highest in Neuchatel, Biel and St. Gallen. Wastewater analysis confirms these findings, except for St. Gallen where substantially lower loads were measured. Similarly to amphetamine use, methamphetamine consumption appears to be more widespread in the northern part of the country.

In both countries, highest MDMA loads were measured in the largest cities, Berlin and Zurich, with the latter having the overall highest figures. Measurements in Munich were similar to those recorded in other larger urban areas (*i.e.*, > 100'000 inhabitants) in Switzerland, while Dortmund and Dülmen showed loads similar to smaller cities in Switzerland. Amphetamine consumption was clearly more prevalent in the northern part of Germany. This is in agreement with national figures which suggest that its consumption is higher in Germany (12-months prevalence: 0.7% for ATS (260)) compared to Switzerland (12-months prevalence: 0.4% for amphetamine and 0% for methamphetamine (173)). In the case of methamphetamine, available data indicate that consumption is centred in some “hotspots”, while other areas included in this study are less affected.

Results from wastewater analysis obtained in this context were in line with the multi-city study reported by the EMCDDA (136,258).

13.5.4 Heroin

The highest offence rates were reported in Lausanne, Bern and Biel, while the highest 6-MAM loads were measured in Zurich and Winterthur. Except for the latter two cities, the picture drawn from wastewater analysis suggests that heroin consumption is quite uniform throughout the country. In agreement with reported offences, Sion and Chur were the cities with the lowest per capita loads. There is evidence that the number of people being apprehended for consumption of highly stigmatized drugs is likely influenced by law enforcement strategies, in particular for heavy opiate users. Thus, classical surveillance systems provide a biased picture of the situation, rendering comparisons difficult.

13.5.5 Consumption estimates

Consumption estimates for each substance and city included in the sampling frame are reported in Table 37. Whilst these figures are affected by various uncertainties, and should

thus be interpreted carefully, they provide a rough idea of the amounts of substances consumed in the investigated areas. As previously discussed, amphetamine seems to be the predominant stimulant drug in Germany, while cocaine seems to be more widespread in Switzerland. Data for Liechtenstein suggest that cocaine is the main stimulant, with levels of use comparable to cities of similar size in Switzerland.

Nationwide consumption estimates for cocaine and MDMA for Switzerland only derive from data collected in larger urban areas over the course of one week. These estimates may thus not correspond to consumption in smaller catchments not included in the study (*i.e.*, less than 40'000 inhabitants), nor may they be representative of consumption throughout the year. Moreover, the excretion rates used for the calculations, which derive from pharmacological studies with a limited number of participants, and the potential degradation of biomarkers in sewers, might further affect the accuracy of the estimates. Nonetheless, these rough estimates allow assessing the order of magnitude of quantities consumed on a national scale and could potentially be used to estimate the associated money turnover.

13.6 Conclusion

Because illicit drug consumption is a complex phenomenon, the indicators considered in this study can only provide a partial perspective of the phenomenon and all suffer from limitations and uncertainties. Survey data may be biased by differential responses due to stigmatisation and hidden behaviours, which are particularly pronounced for heroin and cocaine, whereas cannabis and other stimulants might be less affected. Offence data are obviously biased by law enforcement activities and strategies as well as the visibility of the drug scene and thus, may not be representative of the size of the drug using population. Munich is a clear example: offence rates related to all considered substances were among the highest while prevalence and wastewater data suggest low numbers of users and quantities. Moreover, this type of data does not always allow for distinguishing between users and dealers (who might confess personal use to avoid charges for trafficking). Finally, wastewater analyses allow estimating the amount and type of products consumed by the community, yet they are not capable of estimating the number and type of consumer.

These limitations might explain the dissimilarities observed for cocaine-related data. As a result of its negative connotation and hidden consumption, it is likely more difficult to capture regular and marginalised users with current survey methods. Additionally, drug-related public nuisances influence the activities of law enforcement, making it difficult to draw conclusions from the number of reported offences. On the contrary, an interesting overlap could be highlighted for data related to cannabis, suggesting relatively homogeneous consumption across Germany and Switzerland. The findings support the hypothesis that cannabis consumption is less stigmatised and more widespread, and that current methods and wastewater analysis provide a realistic perspective of the situation. Similarly, a good overlap was observed for ATS-related data. A more widespread recreational use and thus, a limited number of regular/heavy users (difficult to measure) might explain the good agreement between the considered indicators. For Germany, prevalence data was not substance-specific

(*i.e.*, not possible to distinguish between amphetamine, methamphetamine and MDMA) and wastewater analysis provided a valuable tool to highlight geographical differences. Similarly, in Switzerland, where no recent survey was available, wastewater analysis allowed to identify geographical differences in amphetamine and methamphetamine use. Amphetamine was found to be more predominant in Germany and cocaine in Switzerland, confirming the north-south gradient of stimulants use in Europe (*i.e.*, amphetamine being the main stimulant in northern countries, while cocaine is more widespread in the south of Europe (2,262)). Nonetheless, it would be necessary to include additional areas, in particular for Germany, in order to obtain a more precise image of the situation at the national level.

The findings support some of the existing hypotheses about regional features, but also provide additional evidence about geographical particularities. Understanding illicit drug consumption on a national scale remains a difficult task, however, the findings of this research illustrate how the combination of different and complementary data sources allows for obtaining a more accurate picture of the situation. The retrieved information can be used to monitor changes in drug use, both at the national and international scale, identify potential dangers, promote the setup of specific interventions (e.g., targeted surveys, prevention campaigns and/or police actions), understand the structure of drug markets and guide future drug policies. Although wastewater analysis does not provide direct information about users, its ability to provide close to real-time data and its potential integration in existing monitoring programs make it a valuable tool to help understanding illicit drug consumption.

13.7 Acknowledgements

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13.8 Supporting Information

13.8.1 Epidemiological data and police statistics

13.8.1.1 Germany

Epidemiological data for Germany was gathered from various sources. For Berlin, two sources were available: general population survey conducted in 2012 among the population aged 15 to 64 (180) and prevalence estimates based on capture/recapture methods using drug related data (hospital admission, treatment demands, police records and drug-related death) for 2010 and 2011 (181). For Munich, Dresden, Dortmund and Dülmen only data representative of the corresponding Federal State (Bundesland) were available. For Bavaria (Munich) and Saxony (Dresden), figures dated back to 2009 (population aged 18-64) (182,183), while for Northrhein-

Westfalia (Dortmund and Dülmen), only data from 2000 were available (population aged 15-59) (184). Although there might be some differences between urban and rural areas, these were assumed to be representative of the consumption of the considered cities. Cocaine and “crack” were considered together.

Police statistics consisted in the number of registered offences for consumption of illicit drugs reported in the investigated areas during 2014. We obtained this data from police forces of the investigated areas. We then divided the data by the number of inhabitants living in each area so to obtain the number of offences reported per year per thousand inhabitants.

13.8.1.2 Switzerland

Epidemiological data for Switzerland were available as 12-month consumption prevalence for cocaine and cannabis collected via telephone surveys (Continuous Rolling Survey of Addictive Behaviours and Related Risks (264)). Only respondents whose residence was within the catchments were considered to obtain geographically relevant data. To increase sample size, data from four successive waves of the annual survey (2011 to 2014) were compiled. However, it needs to be reminded that the survey was not designed to be representative at the catchment scale. Thus, for drugs with low prevalence, smaller differences between cities might not be statistically significant. Analogous to German data, cocaine and “crack” were considered together as cocaine data.

Similarly to Germany, police statistics consisted in the number of registered offences for consumption of illicit drugs reported per week during 2013. Data was provided by the Swiss Federal Office of Statistics. Only offences perpetrated within the considered catchments were retained. Weekly data were summed and divided by the resident population to obtain an estimate of the yearly number of offences per thousand inhabitants.

13.8.2 Wastewater sampling and analytical procedures

Daily 24-hour composite raw wastewater samples were collected over 7 consecutive days in March 2014. Relevant information for each WWTP catchment was gathered systematically by means of a standardized questionnaire (136,146). Upon reception in the laboratories, samples were spiked with isotope-labelled internal standards, either filtered and extracted immediately on solid-phase extraction cartridges or frozen at $-20\text{ }^{\circ}\text{C}$ until analysis. The collected samples were analysed by four different laboratories using all liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with triple quadrupole analysers (QqQ). The samples were either directly injected by means of large volume injection (LVI) or pre-concentrated by solid-phase extraction (SPE). The analytical procedures were fully validated and participating laboratories passed the inter-laboratory exercises performed in a collaborative study.

13.8.3 Illicit drug loads and back-calculations

All estimates were computed using Monte Carlo simulations using the parameters described in Table 34 and Table 35. An additional uncertainty of $\pm 15\%$ due to sampling was associated to the calculated loads (95,102,157). Following the same procedure as (136), when measured concentrations were below the limit of quantitation (LOQ) of the analytical method, loads were

calculated by taking half of the LOQ. No back-calculations were performed to estimate heroin consumption, because estimates derived from its specific metabolite 6-MAM were shown to be inconsistent (265). Similarly, no back-calculations were made for THC-COOH due to the limited pharmacological data. In the specific case of Berlin, daily loads calculated for each WWTP were added up and divided by the total size of the served population. Data analysis and simulations were computed using R software (R Foundation for Statistical Computing, Vienna). Illustrations were made with Tableau Software (Seattle, WA, USA).

13.8.4 Prevalence of amphetamine-type stimulants consumption in German cities

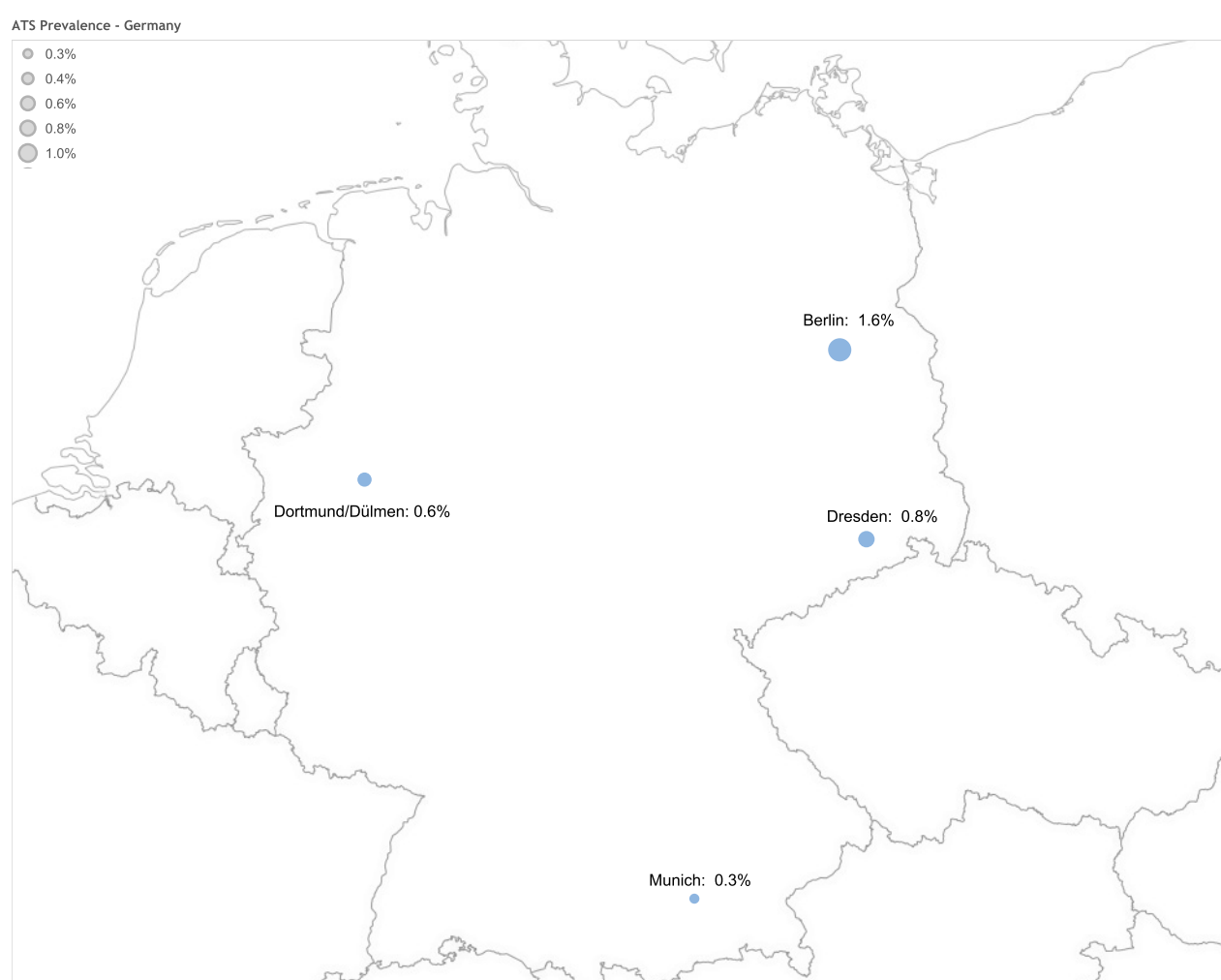


Figure 48: Prevalence data for amphetamine type stimulants for the Federal States of Northrhine-Westfalia (Dortmund and Dulmen), Saxony (Dresden) and Bavaria (Munich).

13.8.5 Heroin consumption offences and 6-monoacetylmorphine loads in Switzerland

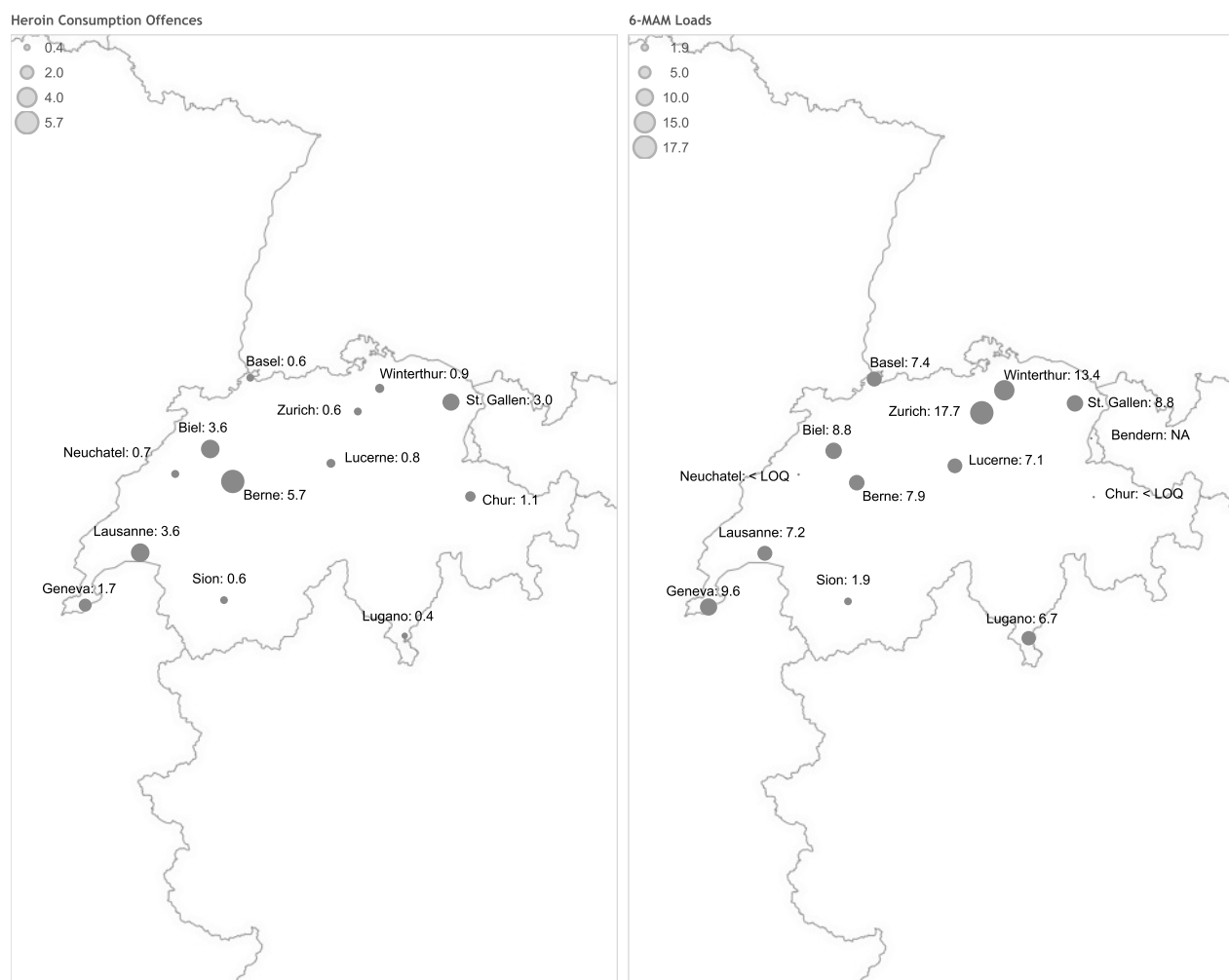


Figure 49 : Heroin consumption offences per thousand inhabitants and population normalised loads [mg.day⁻¹.1000 inhabitants⁻¹] of 6-monoacetylmorphin measured in Swiss cities.

13.8.6 Weekly loads - Germany

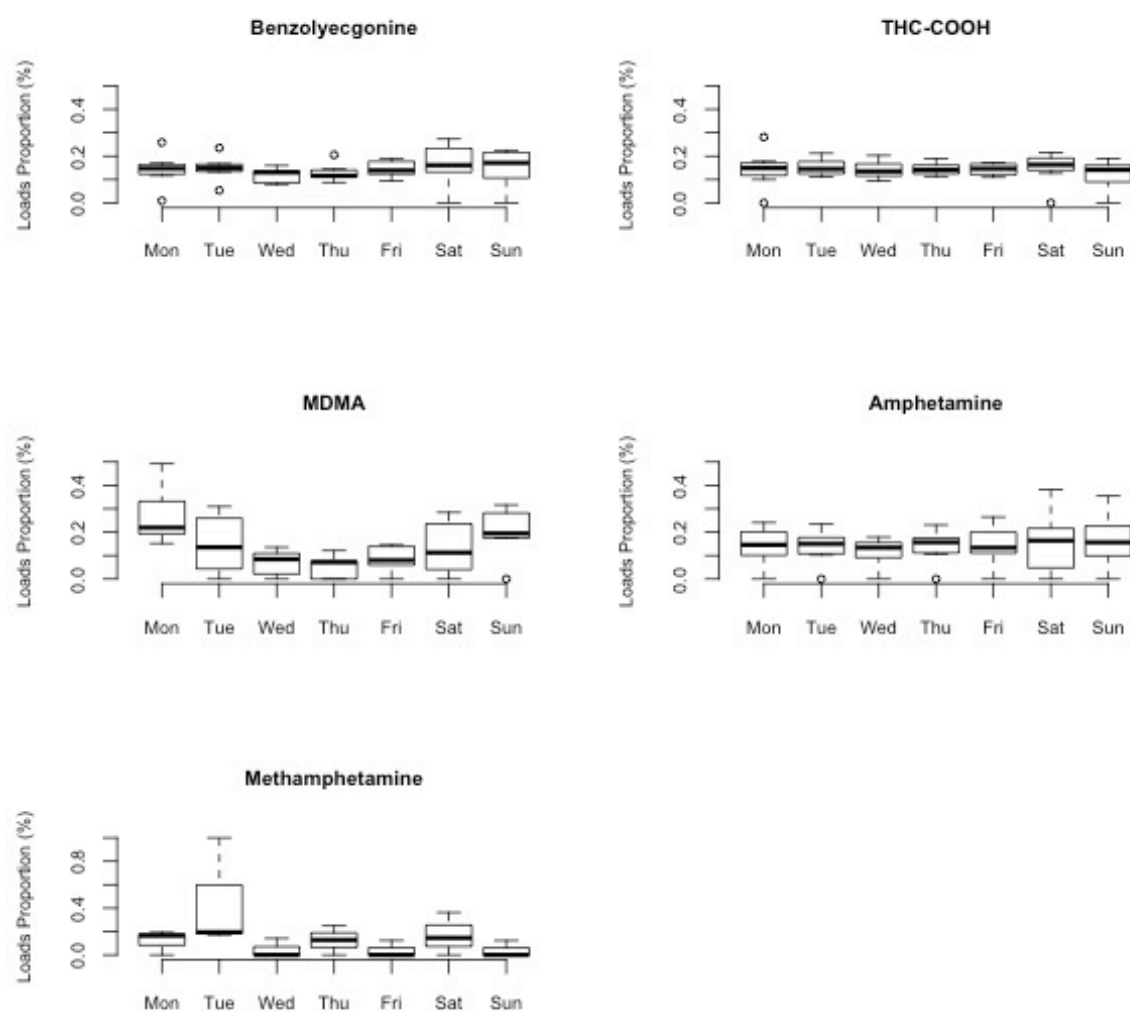


Figure 50: Proportion of illicit drug loads measured during each day of the week in all WWTP in Germany. For each city, the loads measured on each day were divided by the sum of loads measured during the whole week.

13.8.7 Weekly loads - Switzerland

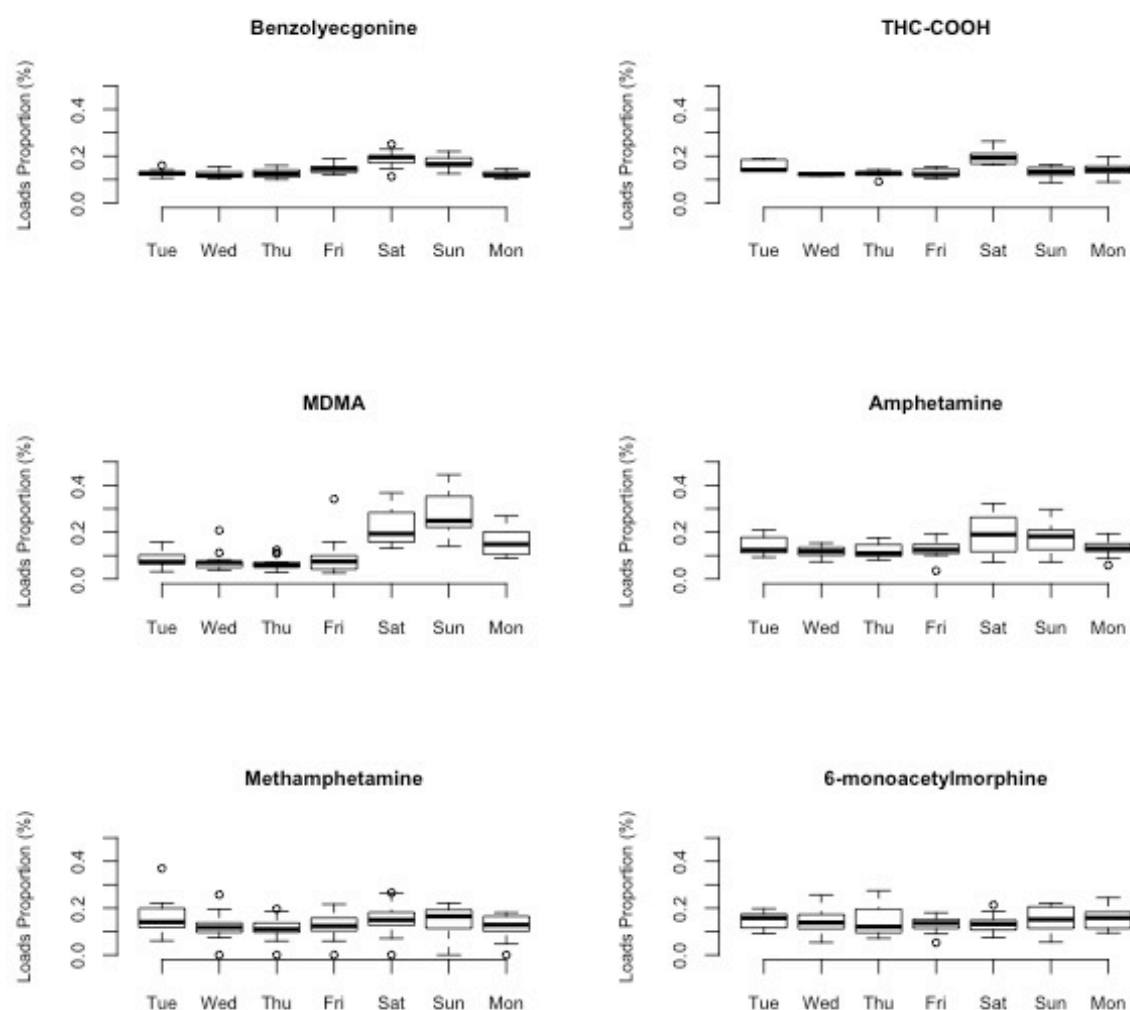


Figure 51: Proportion of illicit drug loads measured during each day of the week in all WWTP in Switzerland and Liechtenstein. For each city, the loads measured on each day were divided by the sum of loads measured during the whole week.

13.8.8 Nationwide estimates

The response variables (i.e., the estimated consumption of cocaine and MDMA) were log-transformed to correct for heteroscedasticity (i.e., unequal variances). A plot of the transformed data is shown in Figure 52. To model the estimated consumption as a function of the size of the population and the day of the week, the following functions were initially considered: linear (1), quadratic (2), square root (3) and cubic (4).

$$y = \beta X + uZ \quad (1)$$

$$y = \beta X + \gamma X^2 + uZ \quad (2)$$

$$y = \beta X + \gamma \sqrt{X} + uZ \quad (3)$$

$$y = \beta X + \gamma X^2 + \delta X^3 + uZ \quad (4)$$

where y is a vector of response variables (i.e., back-calculated amount of substance), β , γ and δ are the coefficients associated to the fixed effect X , which is the size of the population in the investigated catchments. The last two parameters were used only for the mixed effect models: u represents the vector of coefficients associated to the random effect Z , which corresponds to the days of the week (Tuesday to Monday). For all models, it was assumed that the intercept is equal to zero (i.e., if the population is equal to zero, there is no consumption).

For cocaine, ordinary least square models performed better in terms of R^2 , however there was a clear difference in terms of RMSE compared to mixed effect models. In particular the model including the square root of the population provided the best results. Although lower R^2 were obtained compared to ordinary least square regression, this model was selected to extrapolate nationwide consumption figures of cocaine because, a part from showing the lowest RMSE, it accounts for differences observed between days of the week.

For MDMA, the ordinary least squares model including the square root of the population performed better than other models both in terms of RMSE and adjusted R^2 (see Table 39). Mixed effect models did not provide satisfactory results (i.e., high RMSE and low R^2) mainly because in smaller catchments MDMA could not always be detected, thus when these were used to test the models performances large discrepancies were obtained between observed and predicted values.

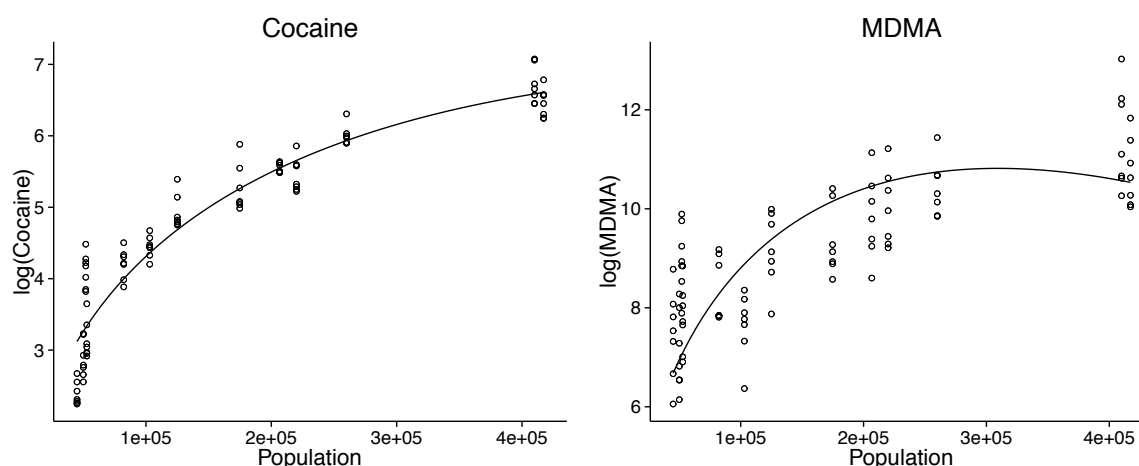


Figure 52: Plot of log-transformed cocaine (left) and MDMA (right) consumed quantities as a function of the size of the population and the fitted models. MDMA data was transformed to milligrams prior to log-transformation to avoid values smaller than 1.

Table 39: Summary of model performances for cocaine and MDMA. The models were computed using a training set from which the data for one of the cities was removed. The latter was then used to test the model and calculate the root mean square error (RMSE) and the adjusted coefficient of determination (R^2). This process was repeated iteratively to use the data of each city once as test set and calculate the average RMSE and R^2 .

<i>Compound</i>	<i>Parameter</i>	<i>Ordinary least squares model</i>			
		<i>Linear</i>	<i>Quadratic</i>	<i>Square root</i>	<i>Cubic</i>
<i>Cocaine</i>	RMSE	227.21	229.99	230.06	230.04
	Adjusted R^2	0.84	0.97	0.99	0.99
			<i>Mixed effect model</i>		
	RMSE	191.41	68.86	60.77	103.03
	Adjusted R^2	0.66	0.66	0.66	0.66
			<i>Ordinary least squares model</i>		
<i>MDMA</i>	RMSE	9338.19	95.15	15.13	31.72
	Adjusted R^2	0.76	0.92	0.99	0.97
			<i>Mixed effect model</i>		
	RMSE	27.14	21.72	21.75	21.34
	Adjusted R^2	0.61	0.61	0.61	0.61

XIV Annexe IV: Integrating environmental and self-report data to refine cannabis prevalence estimates in a major urban area of Switzerland

Been F., Schneider C., Zobel F., Delémont O., Esseiva P.; *Integrating environmental and self-report data to refine cannabis prevalence estimates in a major urban area of Switzerland, submitted.*

14.1 Abstract

Background: Cannabis consumption is a topical subject because of discussions about reviewing current regulations. In this context, having a more comprehensive approach to assess and monitor prevalence and consumption would be highly relevant. The objective of this work was to refine current estimates about prevalence of cannabis use by combining self-report data to results derived from wastewater analysis. **Methods:** Self-report data was retrieved from surveys conducted in Switzerland and Europe. Wastewater samples were collected at the wastewater treatment plant of a city in western Switzerland over a 13 months period. The occurrence of 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH), a specific metabolite of delta-9-tetrahydrocannabinol (THC), was monitored. Bayesian hierarchical models were used to estimate consumption, prevalence and number of cannabis users in the investigated area. **Results:** According to survey data, 12-months prevalence in western Switzerland was estimated to 6.2% of the population aged 15 or older, with an estimated daily cannabis consumption of 1.6 kg.day⁻¹ (at 11.2% purity). The integrative model comprising self-report and wastewater data allowed to significantly reduce the uncertainty in the estimates and suggested a last-year prevalence of 9.5%, with a daily cannabis consumption of 2.9 kg.day⁻¹. **Conclusion:** Although in the same order of magnitude, estimates obtained with the integrative model were almost 40% higher compared to self-report figures. Interestingly, these figures are similar to discrepancies observed when comparing self-reported alcohol consumption and sales or tax data. The suggested integrative model allowed to account for known sources of uncertainty and provided refined estimates of cannabis prevalence in a major urban area of Switzerland.

Keywords: Self-report, wastewater, cannabis, prevalence, Bayesian analysis

14.2 Introduction

In recent years, there has been a rapid increase in number of studies focused on monitoring illicit drug use through wastewater analysis (also referred to as wastewater-based epidemiology (WBE)) at the regional (85), national and international level (136). Due to its widespread use and high prevalence rates (266), cannabis has been among the target substances since the first applications of the approach (86). Better understanding the extent of its use is important for policy makers seeking to hamper criminal activities and their revenues as well as introducing new drug policies. This is particularly important with regards to discussions about legalising its use and regulating the markets (187,188), which are also highly topical in Switzerland (191,267,268).

In WBE, most attention has been given to 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH), one of the metabolites of the major active compound found in cannabis, i.e. delta-9-tetrahydrocannabinol (THC). Yet, the analysis of THC-COOH as well as the estimating consumption, referred to as “back-calculations”, are quite challenging (136). Although THC-COOH has been shown to be stable in sewer conditions over longer periods of time, potential losses during sample preparation steps have been highlighted (118,119,124). These can however be partly accounted for using stable isotope-labelled internal standards. Consumption estimates were initially computed using only data relative to urinary excretion of THC-COOH (46,52), however faecal excretion is known to be the major route by which THC and its metabolites are eliminated from the body (42,47,269). Considering that at pH values commonly measured in wastewater (i.e., 7.5) THC-COOH is expected to be ionized, and thus almost completely dissolved, using only excretion rates relative to urine will overestimate consumption. The relatively low proportion of THC-COOH found to adsorbed onto suspended solids (117,124) further supports that what is excreted via faces will be dissolved in wastewater. Unfortunately, data about faecal excretion of THC-COOH is limited and there is a need for complementary sources of information to which consumption estimates obtained from wastewater analysis could be confronted.

In regards of these observations, the objective of the present work consisted in monitoring the occurrence of THC-COOH in wastewater and combine the collected data to results from epidemiological studies. For this, i) wastewater samples were collected over a period of 13 months at the inlet of the wastewater treatment plant (WWTP) of a city in western Switzerland. ii) The occurrence of THC-COOH was monitored and, through a Bayesian hierarchical model, used to estimate the amounts of cannabis used. ii) An additional model was developed to estimate consumption and prevalence based on survey data. iv) Finally, the two models were combined to obtain a refined estimate of both consumption and prevalence.

14.3 Material and methods

14.3.1 Wastewater sampling and instrumental analysis

Wastewater samples were collected at the inlet of the wastewater treatment plant (WWTP) of the city of Lausanne, in western Switzerland. The WWTP serves the city itself and 15 additional municipalities in its surroundings, for a total population of approximately 226'000 inhabitants (census based). Samples were collected at the influent of the WWTP using an autosampler (6712FR ISCO refrigerated (4°C) autosampler, Teledyne ISCO, Lincoln, NE) over a 15 months period (between October 2013 and December 2014). Twenty-four hour composite samples were collected from Tuesday to Wednesday and from Saturday to Sunday (12PM to 12PM) every second and forth week of the month. A total of 41 (20 week-day and 21 weekend samples) were collected. Details about the procedure used to process the samples are reported elsewhere (76). After processing and extraction, samples were analysed UHPLC system (1290 Infinity, Agilent, Santa Clara, CA, USA) coupled to a triple quadrupole mass spectrometer (5500 QTrap, ABSciex, Ontario, Canada) to determine THC-COOH concentrations. The analytical method was fully validated and additional details about its performances are reported in *Section 14.7.1*.

14.3.2 Modelling and data analysis

To estimate prevalence of cannabis consumption in the investigated population, a Bayesian hierarchical model was implemented. These types of models have the great advantage of allowing the integration of prior knowledge about the parameter(s) to be estimated. Moreover, they allow to compute the posterior distribution of the estimate and quantify its uncertainty (270). When confronted to particularly complex models, where directly sampling from the posterior distribution is not possible, methods such as Markov Chain Monte Carlo (MCMC) simulations are often implemented to estimate model parameters (270,271). This approach was implemented in the context of this work and the resulting model is presented in Figure 53 in the form of a directed acyclic graph (DAG). The latter illustrates the causal relationship between i) the number of cannabis users and their consumption habits (left part of Figure 53); ii) metabolization and excretion of the active ingredient (i.e., THC); iii) the occurrence of drug metabolites in wastewater samples and iv) their analysis (right part of Figure 53). Part of this model is founded on previous works by Jones et al. (147). Modelling, calculations and statistical analysis were carried out using R software (272,273) and WinBUGS (274).

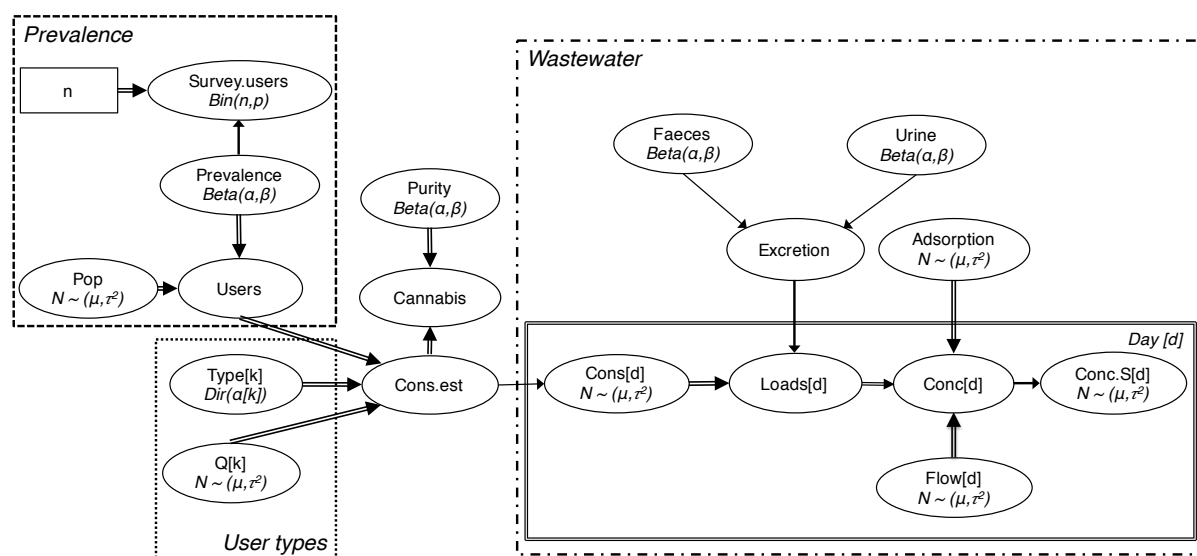


Figure 53: Directed acyclic graph illustrating the hierarchical model used to estimate prevalence of cannabis consumption in the investigated area. Nodes represent stochastic variables (round) and constants (squares). Arrows represent stochastic (single) and logical (double) relationships. *Prevalence*: refers to the modelling of prevalence of cannabis consumption in the investigated area; *User types*: refers to the modelling of user types and their daily consumption; *Wastewater*: refers to modelling of excretion, occurrence in wastewater and analysis of THC-COOH and was founded on previous work by Jones et al. (147). See Table 40 for details about each node.

14.3.3 Modelling wastewater loads and back-calculations

Calculations of THC-COOH loads and back-calculations were carried out using a modified version of the Bayesian hierarchical model suggested by Jones et al. (147) to estimate cocaine consumption. The corresponding part of the model is shown in the frame named *Wastewater* in Figure 53 and the parameters used are described in Table 40.

In the context of this work, THC-COOH excreted via faeces was considered to be fully dissolved in wastewater. This was assumed given the chemical properties of the compound, which is expected to be ionised at pH values measured in wastewater, and the turbulences in sewers which will disintegrate faecal matter and thus further promote dissolution. Yet, experiments have shown that a small portion of THC-COOH load are adsorbed onto suspended solids (i.e., 8.5%) (117). Because these were not analysed in this context, an additional factor was included (i.e., *Adsorption*), thus allowing to estimate total THC-COOH loads (i.e., dissolved + adsorbed).

Annexe IV: Integrating environmental and self-report data to refine cannabis prevalence estimates in a major urban area of Switzerland

Table 40: Summary of parameters used in the model described in Figure 53. d = day, SE= Standard Error, $\tau = 1/SE^2$. Mwm and Mwc = molecular weights of the metabolite (THC-COOH, 344.445 g/mol) and the parent compound (THC, 314.45 g/mol), respectively.

<i>Node</i>	<i>Value/Distribution</i>	<i>Parameter 1</i>	<i>Parameter 2</i>	<i>Description</i>	
Conc.S[d]	Normal ($\mu[d]$, τ^2)	μ = THC-COOH concentration measured in WW sample collected on day d [ng/L]	SE = 25% of measured concentration	Estimate of the actual THC-COOH concentration measured in the collected wastewater samples. SE was estimated through validation of the analytical method	
Conc[d]	$\left(\frac{Loads[d]}{(1 + Adsorption)/Flow[d]} \right) * 1e^9$	-	-	THC-COOH concentration in wastewater in [ng/L]	
Flow[d]	Normal ($\mu[d]$, τ^2)	μ = Total daily flow on day d [L/day]	SE = 12.5% of daily flow	Wastewater flow measurements. SE was provided from the WWTP personnel	
Adsorption	Normal (μ , τ^2)	μ = 8.5%	SE = 2.0%	Portion of total THC-COOH loads expected to be adsorbed onto suspended solids (117)	
Wastewater	Loads[d]	$Cons[d] * Excretion * \frac{Mwm}{Mwc}$	-	-	Estimated daily loads of THC-COOH in [g/day]
	Excretion	<i>Faeces + Urine</i>	-	-	Sum of the excretion of THC-COOH from urine and faeces
	Faeces	Beta (α, β)	μ = 6.7%	SE = 2.9%	Excretion of THC-COOH in faeces. α and β were estimated using the aggregated distribution computed from data reported by Wall et al. (47) and estimates by Khan & Nicell (186)
	Urine	Beta (α, β)	μ = 0.039%	SE = 0.018%	Excretion of THC-COOH in urine. Inverse-variance weighed average estimated from data reported in literature review by Khan & Nicell (186).
	Cons[d]	logNormal (μ , τ^2)	Estimated THC consumption on day d [g/day]	Non-informative priors <i>Unif</i> (0, 1000)	Data was log-transformed to correct for skewness. The average of <i>Cons[d]</i> (μ) is used as an estimate of <i>Cons.est</i>

Annexe IV: Integrating environmental and self-report data to refine cannabis prevalence estimates in a major urban area of Switzerland

Table 40 (continued)

	<i>Node</i>	<i>Value/Distribution</i>	<i>Parameter 1</i>	<i>Parameter 2</i>	<i>Description</i>
	Cons.est	$\sum_{i=1}^k Users * Q[k] * Type[k]$	-	-	Estimated average daily (pure) THC consumption [g/day]
	Purity	Beta (α, β)	$\mu = 11.2\%$	SE = 0.2%	Weighted average and standard error derived from seizure analysis in Switzerland (190). Weighing was based on the number of herb, resin and oil samples analysed. See Supporting Information for further details (Table S1)
	Cannabis	$\frac{Cons.est}{Purity}$	-	-	Estimated amount of cannabis consumed per day (regardless of the form)
	n	2665	-	-	Number of individuals (aged 15 and older) having participated to the Swiss survey (173)
Prevalence	Survey.users	Binomial(n,p)	$n = 2665$	$p = 0.063$	Users having reported cannabis use in the Swiss survey (173)
	Prevalence	Beta (α, β)	$\alpha = 1$	$\beta = 1$	Estimate of last-year cannabis prevalence. Uninformative priors were used
	Pop	Normal (μ, τ^2)	$\mu = 199'209$	SE = 39'214	Population living in the investigated area estimated using ammonium
	Users	$Prevalence * Pop$	-	-	Number of individuals in the investigated area having consumed cannabis at least once per year
User types	Type[k]	Dirichlet($\alpha_1, \alpha_2, \alpha_3, \alpha_4$)	$\alpha =$ see Table 41	-	Number of users in each category according to the Swiss survey (173) (see Table 41)
	Q[k]	Normal (μ, τ^2)	$\mu =$ see Table 42	SE = see Table 42	Average daily amount of THC consumed by user type k . Estimated from (189) (see Table 42)

14.3.4 Modelling excretion of THC-COOH

As shown in Figure 53, total *Excretion* of THC-COOH was assumed to be equal to the sum of urinary and faecal excretion. Other excretion routes (e.g., sweat, oral fluid and hair (42)) were considered negligible. To the authors' knowledge, only the study by Wall et al. (47) reported faecal excretion. In their study, the authors assessed that faecal excretion of THC-COOH after 72h following intravenous and oral administration was 6.1 ± 4.3 and 9.5 ± 3.7 % of the initial THC dose, respectively (n = 9 women and 11 men) (47). However, these administration

routes, and in particular intravenous injection, are uncommon. In an attempt to overcome this issue, Khan & Nicell (186) estimated the proportion of a smoked dose of THC which is expected to be excreted as THC-COOH. By assuming that the metabolic disposition of THC after smoking and intravenous injection are similar, they estimated that between 3.1 and 5% of a smoked THC dose is expected to be excreted as THC-COOH in faeces. To obtain estimates that could be used in this context, an approach based on “modelling expert opinion” (275) was implemented to elicit the parameters of the *Beta* distribution describing faecal excretion of THC-COOH. These methods are generally used to obtain the distribution of specific random variables when there is limited data or when the available information is only based on experts opinions (275). Briefly, from the means and standard deviations reported by Wall et al. (47), *Beta* distributions were computed and random deviates ($n = 1000$) were used to estimate the 10, 50 and 90% quantiles. For the estimates by Khan & Nicell (186), the reported range and midpoint were taken as quantiles. The aggregated distribution of quantiles from both studies was then computed using a method developed by Cook (275–277), assigning equal weights to each initial estimate of THC-COOH excretion. The aggregated distribution was then used as empirical distribution to which a *Beta* distribution was fitted. The latter was used to estimate the mean faecal excretion of THC-COOH after consumption of THC and the associated standard error (SE) (values reported in Table 40). An illustration of the obtained distribution is shown in Figure 54.

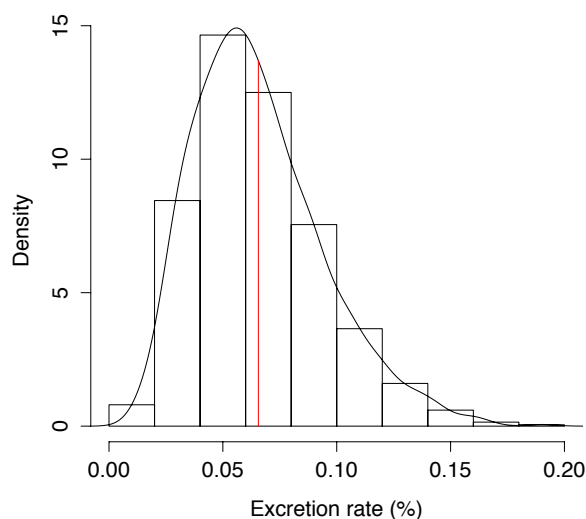


Figure 54: Fitted *Beta* distribution used to model faecal excretion. Blue line: estimated mean excretion rate.

14.3.5 Modelling prevalence of cannabis use from epidemiological data

The following section describes parameters used to model prevalence of cannabis consumption based on epidemiological data (see frame *Prevalence* in Figure 53). Data relevant to prevalence of cannabis consumption in western Switzerland were available from a survey conducted in 2013 (173). Among the 2665 respondents (aged 15 and older), 165 reported having consumed cannabis in the past 12 months, corresponding to a prevalence of

6.2%. The number of users having reported consumption in the past 12 months (*Survey.users*) was modelled as a binomial distribution $B(n,p)$ (n = number of individuals questioned and p = proportion of positive responses). Using the results of the survey, the *Prevalence* was modelled using a *Beta* distribution with uninformative priors ($\alpha = \beta = 1$). The number of cannabis consumers in the investigated area (*Users*) was calculated by multiplying the *Prevalence* by the size of the investigated *Population*. The latter was estimated using a previously developed approach based on the measurement of ammonium in wastewater (76). This approach was preferred to census-based figures as these are static and do not account for eventual fluctuations in the size of the population which can occur during time (e.g., commuters, holidays). Ammonium is mainly introduced in wastewater through the hydrolysis of urea and can thus be considered an indirect marker of urine content (38,40,148). Using so called “population equivalents” (amounts of ammonium generated per person per day), it is possible to estimate the size of the population contributing to the generated wastewater (76). In this context, the size of the population was estimated to $199'209 \pm 39'214$ (SE) individuals. According to demographic data, the proportion of the population aged 15 or older, makes up for 84.5% of the total population in the investigated area (254). Thus, the size of the relevant population considered was estimated to $169'128 \pm 33'292$ (SE) individuals and was assumed to be normally distributed (see *Pop* in Figure 53).

14.3.6 Modelling user types from epidemiological data

The following section describes parameters and estimates used to model the types of users and the amounts of cannabis consumed per user types (see frame *User Types* in Figure 53 and Table 40) based on data available in the literature.

Cannabis users have often been separated into categories based on their yearly or monthly consumption frequency (189). In the Swiss survey, four categories were used based on past months consumption (173), as shown in Table 41. The number of individuals falling in each category was calculated using the total number of questioned users. These were taken as parameters of the Dirichlet distribution ($Dir(\alpha_1, \dots, \alpha_4)$) used to model the proportion of users in each category (cf. *Type[k]* in Figure 1). Uninformative priors were attributed to the parameters of the distribution ($\alpha_1, \dots, \alpha_4 = 1$), which were then updated using the number of users reported in Table 41.

Table 41: Categories of cannabis users as reported by Gmel et al. (173). The number of users in each category was calculated from the total number of respondents (i.e., 304) and the reported proportions. The total number of chipper, occasional, regular and intensive users were used to model the proportion of cannabis users using a Dirichlet distribution $Dir(\alpha_1+1, \dots, \alpha_4+1)$. a) The terminology by van Laar et al. (189) was adopted here; b) reported figures were rounded up.

^a Category	Days of use (past month)	Proportion	^b Number of users
Chipper	1-3	43.3%	132
Occasional	4-9	24.6%	75
Regular	10-19	13.5%	41
Intensive	≥ 20	18.6%	57

The next step in the modelling approach consisted in estimating the amount of cannabis used by each type of user. Unfortunately, these estimates are not available for Switzerland and figures derived from studies conducted in other countries were considered. In their extensive report, van Laar et al. (189) used different studies as well as a web-survey to estimate the annual consumption of the different type of users in Bulgaria, Czech Republic, Italy, the Netherlands, Portugal, Sweden and England and Wales. In their report, the authors also adopted a four group classification based on the number of days of use per year (i.e., 0-10, 11-150, 151-250, > 251 days of use per year). Moreover, they also reported the preferred form of cannabis (i.e., resin, herb or both) used by the respondents for each country and category. Before this data could be used in this context, two assumptions had to be made: i) the classification of user types in the Swiss survey was considered to be equivalent to the one used by van Laar et al. (189), although in the latter these were based on consumption frequency over one year; ii) only figures for Italy, the Netherlands and Portugal were retained as consumption patterns in these countries were expected to be most similar to Switzerland. The daily consumption per user type (see $Q[k]$ in Figure 53) was estimated by taking the reported yearly consumption (5% trimmed mean) and dividing it by 365.25 days (consumption was assumed to be homogeneously distributed throughout the year). The obtained figures were weighted to account for the preferred form of cannabis used and the average potency of herb and resin in the reference countries (278). Results are shown in Table 42.

Table 42: Estimated daily cannabis consumption in terms of pure THC (cf. Q_k in Figure 53) for each category and reference country. The daily consumption per category and preferred form was calculated by multiplying the proportion of users who prefer one or both forms by the annual consumption (as reported in (189)) and then dividing through 365.25 days. The standard error was calculated as the standard deviation of estimate for each category divided by the square root of the number of countries considered.

		<i>Estimated daily THC consumption [mg/day]</i>			
	<i>Preferred form</i>	<i>Chippers</i>	<i>Occasional</i>	<i>Regular</i>	<i>Intensive</i>
<i>Italy</i>	<i>Resin</i>	0.04	0.44	6.48	23.56
	<i>Herb</i>	0.14	1.16	16.70	61.20
	<i>No preference</i>	0.08	0.61	6.73	27.82
	Total	0.26	2.22	29.91	112.58
<i>Netherlands</i>	<i>Resin</i>	0.05	0.48	3.31	9.02
	<i>Herb</i>	0.05	0.44	5.58	33.48
	<i>No preference</i>	0.06	0.40	2.56	7.54
	Total	0.17	1.33	11.45	50.05
<i>Portugal</i>	<i>Resin</i>	0.07	1.18	13.32	48.32
	<i>Herb</i>	0.06	1.02	7.19	6.98
	<i>No preference</i>	0.08	0.43	1.34	5.16
	Total	0.21	2.63	21.85	60.47
Mean		0.21	2.06	21.07	74.37
Standard Error		0.03	0.39	5.34	19.34

14.4 Results

14.4.1 Wastewater data – THC-COOH loads and back-calculations

Loads of THC-COOH in wastewater were computed as described previously (see *Section 14.3.3*) and are illustrated in Figure 55. It should be noted that these do not represent the amounts of THC (or cannabis) consumed, but only reflect the occurrence of THC-COOH over time in the investigated area.

Annexe IV: Integrating environmental and self-report data to refine cannabis prevalence estimates in a major urban area of Switzerland

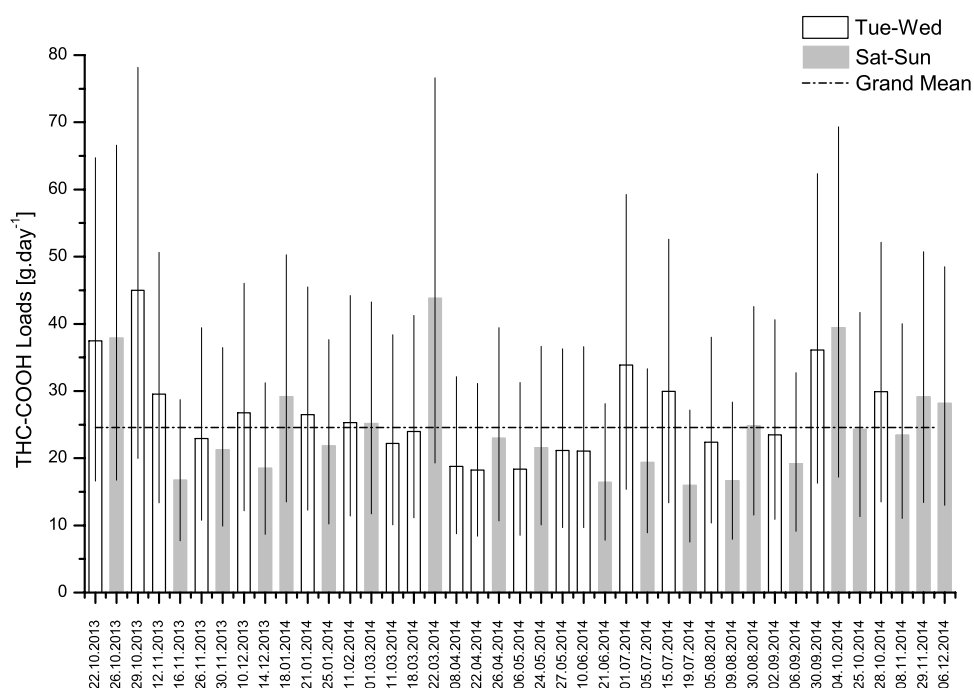


Figure 55: Estimated THC-COOH loads over the sampling period. Samples collected between Tuesday-Wednesday and Saturday-Sunday are shown in white and grey, respectively. The vertical lines show the upper and lower 95% credible intervals (CI). The dashed line shows the average loads.

As can be seen, no particular trend is observable in the data and THC-COOH loads do not seem to substantially deviate from the average value of $24.6 \text{ g}\cdot\text{day}^{-1}$. Moreover, no significant difference could be highlighted between loads measured during week-days and weekends (Wilcoxon rank-sum test p -value = $0.248 > 0.05$).

The amounts of THC consumed, based solely on data related to wastewater analysis, were back-calculated using the model described in Figure 53 (frame *Wastewater*) and Table 40. The obtained results are reported in Table 43. As can be seen, the average daily consumption of THC was estimated at 352.1 g of pure THC (range: 126.7 - $1290.8 \text{ g}\cdot\text{day}^{-1}$). If the average purity of seizures is included (i.e., $11.0 \pm 0.2\%$), this would give, as first approximation, an average cannabis consumption (regardless of the form) of $3.8 \text{ kg}\cdot\text{day}^{-1}$ for the city of Lausanne and its metropolitan area (range: 1.1 - $11.5 \text{ kg}\cdot\text{day}^{-1}$).

Table 43: Results for estimation of the various parameters linked to cannabis consumption using the model described in Figure 53. The number of users and prevalence refer to past year consumption. CI = credible interval. a) Figure derived from survey data (173).

<i>Model</i>		<i>Average daily THC consumption [g.day⁻¹]</i>	<i>Average daily cannabis consumption [kg.day⁻¹]</i>	<i>Number of users</i>	<i>Prevalence</i>
Wastewater data only	<i>Estimate</i>	352.1	3.8	-	-
	<i>95% CI</i>	126.7 - 1290.8	1.1 - 11.5	-	-
Epidemiological data only	<i>Estimate</i>	182.8	1.6	10520	^a 6.2%
	<i>95% CI</i>	85.2-312.1	0.7-2.8	6'277-15'000	5.3-7.1%
Epidemiological and wastewater data	<i>Estimate</i>	321.7	2.9	17090	9.5%
	<i>95% CI</i>	191.5-493.1	1.7-4.4	11'490-23'250	8.4-10.6%

14.4.2 Epidemiological data

Using solely epidemiological data related to prevalence of cannabis use and categories of users described previously, the expected consumption, in terms of pure THC and cannabis as well as the number of users were estimated using the right part of the model described in Figure 53. Results are reported in Table 43. As can be seen, the daily THC consumption was estimated at 182.8 g.day⁻¹ (range: 85.2-312.1 g.day⁻¹) which corresponds to an average cannabis consumption of 1.6 kg.day⁻¹ (range: 0.7-2.8 kg.day⁻¹). In terms of consumers, referred here as to individuals who consumed cannabis at least once in the past 12 months, 10'520 users (aged 15 or older) were estimated, with a range going from 6'277-15'000.

14.4.3 Combining epidemiological and wastewater data

Using the full model described in Figure 53, epidemiological and wastewater data were combined to compute the updated (posterior) estimates of consumption, number of users and prevalence (see frames *User Types* and *Prevalence* in Figure 53). The results are reported in the last row of Table 43 and a plot of the prior and posterior estimates is shown in Figure 56. As can be seen, the updated estimate of pure THC consumption is now 321.7 g.day⁻¹ (range: 191.5-493.1 g.day⁻¹) or, in terms of cannabis, 2.9 kg.day⁻¹ (range: 1.7-4.4 kg.day⁻¹). This corresponds to an increase of approximately 43% compared to survey-based estimates. In terms of users, these were estimated at 17'090 individuals (range: 11'490-23'250), 38% higher compared to the previous figures. Prevalence of cannabis use in the population aged 15 or older was estimated at 9.5% (range: 8.4-10.6%), in other words an average increase of 35% compared to survey-based estimates.

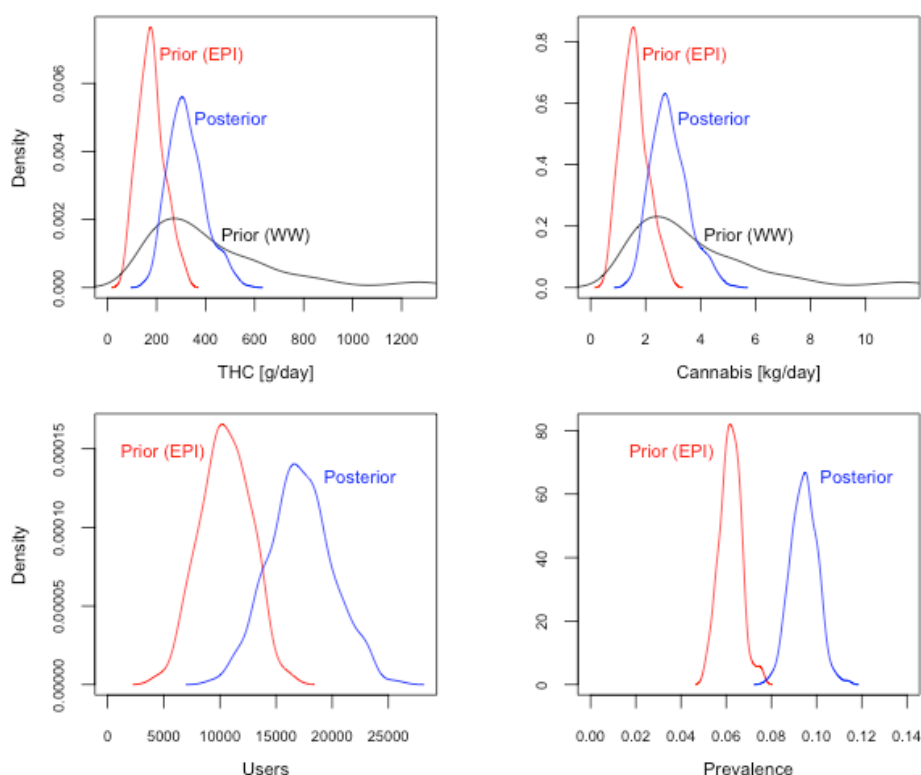


Figure 56: Outputs of the MCMC simulations using epidemiological (Prior (EPI), red curves), wastewater (Prior (WW), black curve) and the combination of epidemiological and wastewater data (Posterior, blue curve).

14.5 Discussion

14.5.1 Temporal analysis

Analysis of THC-COOH loads over the sampling period shows that occurrence of this metabolite is stable, suggesting a steady consumption of cannabis during the sampling campaign. This is also supported by the fact that no difference could be highlighted between loads measured during week-days and weekends, in agreement with previous studies (46,76,279). These findings are interesting considering the modification of drug policies related to cannabis use in Switzerland that occurred on October 1st 2013. New regulations stipulate that consumption of cannabis, and in some regions also possession of up to 10 grams, are to be punished by an administrative fine, instead of being criminally prosecuted (191). Thus, if it is assumed that changes in consumption would occur gradually and that there was no abrupt increase between the 1st and 22nd October (day the first sample was collected), the available data suggests that consumption throughout 2014 has remained stable. This is particularly interesting from a drug policy perspective as it indicates that the relaxation of previous legislation did not promote a sudden increase in consumption in the investigated area. Hence, wastewater analysis proves to be a useful and complementary indicator which could help to rapidly assess whether consumption has increased. In a global context, where current policies about cannabis use are being rethought or undergo scrutiny (188,267), WBE

would be a useful approach to assess the immediate and long-term effects that these will have on consumption.

14.5.2 Consumption and prevalence estimates

When considering results obtained only from wastewater data, it can be seen that quite large credible intervals are obtained from the model (see Table 43 and Figure 56). Although being in the same order of magnitude, the estimated mean consumption is almost twice as high as what was expected from survey data. The difference between the two estimates could be due to the problem of underestimating prevalence, which can be encountered with surveys (187). Yet, another explanation could be the difference in prevalence between urban and rural areas. In fact, the initial figure used here (i.e., 6.2% 12-months prevalence), representative of western Switzerland, does not distinguish the type of agglomeration. However, at the national level, differences in last-year prevalence between urbanised (7.0%) and rural (4.8%) have been highlighted (173). Concerning quantities of cannabis consumed, wastewater and survey data suggest that the daily consumption is somewhere between 0.7 and 11.5 kg.day⁻¹. Nonetheless, because of the wide range, it remains difficult to assess the extent of cannabis use when the two indicators are considered separately.

Estimates obtained using the full model including both survey and wastewater data are still higher than those expected from survey data. However, the credible intervals could be substantially reduced compared to estimates based solely on wastewater data. According to seizures made in Switzerland, herb is the major form of cannabis (89% and 93% of amounts seized at the regional and national level, respectively) (280,281). Thus, the estimated consumption of 321.7 g.day⁻¹ of pure THC (or 2.9 kg.day⁻¹ of approximately 11.2% pure cannabis) is likely due to the consumption of cannabis in herbal form. If it is assumed that consumption is constant throughout the year, which seems to be the case based on the measured THC-COOH loads, the yearly consumption of cannabis in the metropolitan area of Lausanne would correspond to approximately 1.0 metric ton (range: 0.6 – 1.6). When focusing on prevalence, the model estimated that on average 9.5% (range: 8.4-10.6%) of the investigated population aged 15 or more used cannabis at least once a year, which would correspond to 17'090 individuals, based on the distribution of user types depicted in Table 41. If the proportions of users in the different categories are assumed to be realistic, approximately 7400 users would be chippers, 4204 occasional, 2307 regular and 3179 intensive. The latter two groups would consume almost 97% of the total estimate. However, these figures should be interpreted carefully, in particular because of the difficulties of measuring heavy users with general population surveys. Thus, the real proportion of users classed as intensive could potentially be higher than the one considered here. Globally, it can however be stated that including wastewater data in the model resulted in a significant increase in the estimated prevalence of cannabis use compared to survey-based figures (i.e., between 26 and 58% higher), simultaneously reducing the uncertainty around the estimates. Interestingly, these figures are similar to discrepancies observed when comparing self-reported alcohol consumption and sales or tax figures, with the former being approximately 40-60% lower than

the latter (192,193). Potential explanations for underreporting in the context of alcohol consumption have been associated to the difficulty of including heavy drinkers, recall bias and inaccurate estimates of past intake, to cite a few (192,282). Some of these factors are likely valid also to explain the underreporting of cannabis use.

As discussed previously, analysis of THC-COOH in wastewater still remains challenging, in particular due to the potential losses which occur during sample preparation. Although having been partially accounted for both during the analysis and in the modelling step, there is still a chance of underestimating total THC-COOH loads found in wastewater. Estimation of excretion rates also remains a delicate aspect due to the limited number of studies available, in particular for the faecal route. After including survey data into the model, the faecal excretion rate of THC-COOH was estimated to 7.6% (range: 4.6-12.0%), which, although being slightly higher, is the same order of magnitude as the initial estimates. Regarding excretion via urine, posterior estimates were also in line with initial figures (i.e., 0.039%, range: 0.012-0.08%). While there still is a need for additional pharmacodynamic studies, which will allow to better assess the faecal excretion of THC-COOH, there is evidence that the obtained estimates might be realistic representations of the actual metabolism.

In conclusion, measuring THC-COOH in wastewater has been shown to be a useful approach for long-term monitoring of cannabis consumption, a highly topical subject in a global context where current drug policies are being scrutinised. However, precisely estimating consumption and prevalence using survey or wastewater data alone has been shown to be a challenging task due to the various bias and sources of uncertainty affecting these indicators. Still, by trying to take into account the known sources of uncertainty and combining existing and relevant information, it was possible to strengthen the findings and overcome some of these limitations. While still being subject to various sources of uncertainty, the obtained figures allow to refine current estimates about the prevalence of cannabis use in an urbanised area of Switzerland. The presented approach and its findings highlight the utility of triangulating results from wastewater analysis with other, highly relevant, sources of information about illicit drug use. Furthermore, a similar strategy could be implemented to evaluate the prevalence of other substances as well as the structure of consumption (e.g., typology of users and their respective consumption).

14.6 Acknowledgements

The authors would like to thank the city of Lausanne and the personnel of the wastewater treatment plant for allowing this research. Particular thanks go to Matteo Gallidabino and Emanuele Sironi, from the Ecole des Sciences Criminelles at the University of Lausanne, for their precious advices in the statistical analysis. Furthermore, the authors would like to thank Christoph Ort, from the Swiss Federal Institute of Aquatic Science and Technology, for his valuable comments on the manuscript.

14.7 Supporting Information

14.7.1 Wastewater sampling and analysis

The methodology used in this study to analyse wastewater samples was part of a broader analytical method previously described in Been et al. (76,85).

14.7.1.1 Chemicals

Standards of 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH) were purchased as calibrated solutions (1 mg/mL in methanol) from Lipomed AG (Arlesheim, Switzerland). Deuterated (d_3) analogues of the target compound were purchased as calibrated solutions (0.1 mg/mL in methanol) from Lipomed AG (Arlesheim, Switzerland). Stock solutions were conserved in the dark at -20°C . Hydrochloric acid (37%) and ammonium hydroxide ($\geq 25\%$) were TraceSelect® grade (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). Water, formic acid, methanol and acetonitrile were Ultra Chromasolv® grade (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland).

14.7.1.2 Wastewater sampling

Focus of the present study is the city of Lausanne and its metropolitan area, with a population of approximately 226'000 inhabitants, served by one wastewater treatment plant (WWTP). The investigated sewer system is predominantly gravity-drained and the average residence time of wastewater is approximately 2h (240). Samples were collected at the influent of the WWTP using an autosampler (6712FR ISCO refrigerated (4°C) autosampler, Teledyne ISCO, Lincoln, NE) between October 2013 and December 2014. 24h composite samples were collected between Tuesdays and Wednesdays and between Saturdays and Sundays, from 12PM to 12PM, as described in Been et al. (76,265). Briefly, 65mL of wastewater were collected every 5 minutes in 24 1L PP bottles to which $\text{Na}_2\text{S}_2\text{O}_5$ had been previously added (concentration of 2 g/L) (112). A total of 41 samples were collected. Flow measurements (in m^3/s), measured at the influent and averaged over 5 minutes, were provided by the WWTP personnel.

14.7.1.3 Sample preparation and analysis

Immediately after collection, hourly samples were mixed together in a volume proportional manner to obtain a 150mL 24h volume-proportional composite sample. Samples were then filtered using $0.7\mu\text{m}$ nitrocellulose filters (type GF/F, Whatman, GE Healthcare) and acidified to pH 2 using 37% hydrochloric acid (hydrochloric acid (37%)) and spiked with deuterated standards (25 ng/L). Automated solid-phase extraction was carried out immediately after collection and processing the samples, as described in Been et al. (76). Extraction was carried out using Oasis MCX cartridges (3cc, 60mg, Waters, Milford, MA, USA). If the samples were not immediately analysed, dried cartridges were stored in the dark at -20°C until analysis. Elution was performed shortly before analysis using methanol and methanol with 5% of ammonium hydroxide. 0.5mL of the eluate were then evaporated to dryness at room temperature under a gentle stream of nitrogen. The dry residues were then reconstituted in 1

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mL of water (0.1% of formic acid) and methanol (90:10, v/v). Elution was carried out shortly before analysis.

14.7.1.4 Instrumental analysis

Analyses were performed on 1290 Infinity UHPLC (Agilent, Santa Clara, CA, USA) coupled to a 5500 QTrap triple quadrupole mass spectrometer (ABSciex, Ontario, Canada) interfaced with an electrospray ionization (ESI) operated in negative mode. Details about the mass spectrometry parameters are shown in Table 45. The parameters of the chromatographic method can be found in (265).

Table 44: Summary of mass spectrometric parameters.

	R_t	Parent [m/z]	Fragment [m/z]	DP (Volt)	CE (Volt)
<i>THC-COOH</i>	5.32	343.2	299.3 245.1	-45 -45	-28 -35
<i>THC-COOH-d3</i>	5.32	346.1	302.2	-47	-31

Calibration was carried out in the range 100 to 1500 ng/L. Accuracy and intermediary precision were determined using blank drinking water spiked with the target compounds at two different concentration levels (250 and 1250 ng.L⁻¹), extracted and analysed at three consecutive days. Method detection limit (MDL) was determined as the lowest analyte concentration giving a signal-to-noise ratio of three, as well as as correct ratio between the signal of the molecular ion and the fragment ion. The method quantification limit (MQL) was set as the concentration of the lowest level used in the calibration curve (i.e., 100 ng/L). Performances of the extraction method were determined in wastewater samples spiked with deuterated standards (n = 3) before and after extraction, following the procedure proposed in (207,208). Briefly, each sample was split into two aliquots, one of which was spiked before and the other after extraction (the latter representing 100% recovery). The ratio of the signal of the deuterated standard in the two aliquots was used to calculate the recovery. A summary of the methods performances is reported in Table 45.

Table 45: Summary of the validation results. MDL: method detection limit; MQL: Method quantification limit.

	R^2	MDL [ng/L]	MQL [ng/L]	%Recovery (SD)	Concentration [ng/L]	Accuracy	Precision
<i>THC-COOH</i>	0.9998	10	100	46.3 (11.8)	250	29.5%	19.0%
					1250	22.3%	21.7%

14.7.2 Cannabis purity

Purity of cannabis seizures analysed in Switzerland during 2014 was retrieved from annual statistics reported by the Swiss Society of Legal Medicine (190). Results are reported in Table 46.

Table 46: Summary of cannabis seizures analysed by Forensic Laboratories in Switzerland in 2014 (190).

SD: Standard deviation, as reported in (190); SE: Standard error, SD/\sqrt{n} with n being the number of seizures analysed.

<i>Form</i>	<i>Number of seizures analysed</i>	<i>Mean purity</i>	<i>SD</i>	<i>SE</i>
<i>Fresh cannabis with flowers</i>	202	8.3%	5.6%	0.4%
<i>Marijuana and flowers</i>	539	11.6%	5.6%	0.2%
<i>Hashish</i>	61	17.0%	10.7%	1.4%
<i>Oil</i>	4	21.4%	27.5%	13.8%

The average purity of cannabis (regardless of the form) and the corresponding standard error were computed as follows:

$$\bar{x} = \sum_{i=1}^j w_i \mu_i \quad \text{Eq. 2}$$

$$SE = \sqrt{\sum_{i=1}^j w_i^2 SE_i^2} \quad \text{Eq. 3}$$

where \bar{x} is the weighted average, j is the number of cannabis forms considered (i.e. 4), μ_i is the average purity form i (see *Mean purity* in Table 46), SE is the standard error (see *SE* in Table 46) and w_i for the form i calculated as

$$w_i = \frac{n_i}{\sum_{i=1}^j n_i} \quad \text{Eq. 4}$$

where n_i is the the number of seizures analysed for form i .

XV Annexe V: Können Abwasseranalysen der Polizei helfen, Drogenmärkte besser zu verstehen?

Been F., Schneider C., Delémont O., Esseiva P.; *Können Abwasseranalysen der Polizei helfen, Drogenmärkte besser zu verstehen?*, Kriminalistik, 2015, 11, 628-634.

15.1 Abstract

In past years, there has been a substantial improvement in the techniques used for the analysis of illicit drug and their metabolites in wastewater. The present article discusses the uses and limits of the technique for strategic and operative purposes in the field of criminal justice.

15.2 Einleitung

Die Abwasseranalytik ist eine vielversprechende Methode zur Analyse von Drogenmärkten. Im Gegensatz zu herkömmlichen Methoden zur Erfassung des Drogenkonsums – zum Beispiel Umfragen oder Extrapolation aufgrund von Sicherstellungen und Anzeigen – sind Schätzungen basierend auf Abwassermessungen weder vom Willen und der Fähigkeit von Drogenkonsumierenden ihr Konsumverhalten in Umfragen wahrheitsgetreu wiederzugeben, noch von der Intensität oder den Prioritäten der Polizeiarbeit abhängig. Abwasseranalysen versprechen die Schaffung eines vom räumlichen und sozialen Kontext unabhängigen, über die Zeit hinweg vergleichbaren Datensatzes, der es nicht nur ermöglicht, Veränderungen im Konsumverhalten nach zu verfolgen, sondern auch Schätzungen über die in einer Stadt oder einem Land konsumierte Menge einer illegalen Substanz zu entwickeln.

Entsprechend enthusiastisch wurden (zumindest in der Schweiz) in den vergangenen zwei Jahren Veröffentlichungen von Forschungsergebnissen aus der Abwasseranalytik in der Öffentlichkeit und bei Strafverfolgungsbehörden aufgenommen. In einigen Kreisen scheint sich die Erwartung zu entwickeln, dass anstelle der bisherigen „weichen“ – sprich als wenig zuverlässig und lückenhaft empfundenen – Umfragedaten endlich „harte“ Fakten treten würden, wenn es um die Schätzung der Grösse von Drogenmärkten geht.

Was hinter den aus der Abwasseranalytik gewonnenen Daten steckt und welche Aussagekraft sie tatsächlich haben, wird außerhalb von Fachkreisen jedoch selten thematisiert. Dieser Artikel diskutiert diese Grundlagen und argumentiert, dass zumindest ein grundlegendes Verständnis der technischen und statistischen Grundlagen der Abwasseranalytik für die Interpretation der daraus entstandenen Daten unabdingbar ist. Wenn ein solches vorhanden ist, lassen sich die Daten selbst, die mit ihnen verbundenen Unsicherheiten und ihr Beitrag zum polizeilichen Wissen über Drogenmärkte besser einschätzen. Dies wird im Artikel anhand eines Datensatzes zu Kokainrückständen im Abwasser der Schweizer Stadt Lausanne in einer praktischen Anwendung aufgezeigt. Der Artikel kommt aufgrund dieses Beispiels zum Schluss, dass die Anwendungsmöglichkeiten der Abwasseranalytik für polizeiliche Zwecke

gegeben sind. Eine wissenschaftliche Auseinandersetzung mit den Daten und ihren Grenzen ist aber unabdingbar. Nur so können aus dem Abwasser gewonnene Daten einen Beitrag zu besserer Polizeiarbeit leisten.

15.3 Technische und statistische Grundlagen der Abwasseranalytik

15.3.1 Grundlage

Die Untersuchung des Drogenkonsums anhand von Abwasseranalysen basiert auf der grundlegenden Erkenntnis, dass von Menschen eingenommene Substanzen, früher oder später als körperliche Abbauprodukte (sogenannte Metaboliten) in der Kanalisation enden – zumindest wenn im Untersuchungsgebiet ein Abwassersystem existiert. Konsumierte Substanzen werden vom menschlichen Körper adsorbiert, metabolisiert und schlussendlich wieder ausgeschieden. Dabei wird ein Teil der eingenommenen Substanz in ihrer ursprünglichen Form ausgeschieden und ein Teil in Form von Metaboliten. Da der größte Teil der Ausscheidung von eingenommenen Substanzen über Urin und Fäkalien erfolgt, finden sich diese Stoffe im Abwasser wieder. In diesem Sinne kann das Abwasser als eine zusammengefasste, repräsentative Probe des Konsums von Substanzen jener Menschen angesehen werden, die an ein Kanalisationssystem angeschlossen sind (32). Die Gewinnung von Informationen über die Art und Menge konsumierter Substanzen wird als abwasserbasierte Epidemiologie (*wastewater-based epidemiology*) bezeichnet.

15.3.2 Probenahme

Um aus dem Abwasser repräsentative Rückschlüsse über den Substanzkonsum der an der entsprechenden Kanalisation angeschlossenen Bevölkerung ziehen zu können, muss bei der Ziehung der Abwasserproben ein festgelegtes Verfahren angewendet werden.

Die Probennahme wird meistens am Zulauf der Kläranlage vorgenommen, weil dort das Abwasser noch unbehandelt ist. Zur Probennahme werden automatische Probenehmer eingesetzt. Diese bestehen in erster Linie aus einer Pumpe, mit welcher das Abwasser aus der Kanalisation gesogen wird und einem Kühlschranks, in dem die Proben bei 4°C aufbewahrt werden. Die für diesen Artikel verwendeten Daten basierten auf sogenannten Mischproben. Solche Proben werden über einen Zeitraum von 24 Stunden zu verschiedenen Zeitpunkten der Kanalisation entnommen und sind repräsentativ für den Durchfluss in der Kanalisation während eines Tages. Grundsätzlich muss bei den meisten Probenahmen mit Unsicherheiten gerechnet, die generell auf $\pm 15\%$ geschätzt werden (76,95,146,157,283). Eine nicht optimale Probenahme kann aber drastische Konsequenzen auf die Zuverlässigkeit der Messungen haben (95,100,146)

15.3.3 Proben Vorbereitung und Analyse

Nach der Einsammlung werden die Proben behandelt, um sie für die Analyse vorzubereiten. Dazu werden Schwebestoffe aus dem Wasser entfernt und der pH-Wert wird justiert. Für gewisse Anwendungen werden die Proben zusätzlich verdünnt.

Flüssigchromatographie mit Massenspektrometrie-Kopplung ist die am häufigsten verwendete Methode um Drogenrückstände (als Drogenrückstände werden sowohl Metaboliten als auch unveränderte Substanzen im Abwasser bezeichnet) im Abwasser zu analysieren. Da keine Messmethode absolut präzise ist, existieren Unsicherheiten bezüglich der gemessenen Mengen. Diese Unsicherheiten hängen nicht nur von der Messmethode ab, sondern auch von den Substanzen bzw. den Metaboliten, deren Vorkommen im Abwasser gemessen werden soll. Die Benutzung von zertifizierten isotoptenmarkierten Standards für die gemessenen Substanzen sowie die Teilnahme an Leistungstests zwischen verschiedenen Laboren erlauben es aber, die entstehenden Unsicherheiten zu schätzen bzw. die Genauigkeit und Präzision der Methode zu testen.

15.3.4 Rechnung von Substanzfrachten

Nachdem die Abwasserproben eingesammelt, behandelt und analysiert wurden, können die sich im Abwasser befindlichen Frachten (in Gramm oder Milligramm pro Tag) einer Substanz mit der Formel 4 berechnet werden:

$$\mathbf{Frachten [g/Tag]} = \frac{\mathbf{Konz [ng/L]*Q [L/Tag]}}{10^9} \quad \mathbf{Formel 3}$$

In der Formel 4 ist *Konz* die Konzentration einer Substanz, wie sie in der Probe gemessen wurde und *Q* ist der tägliche Durchfluss, der beim Zulauf der Kläranlage gemessen wurde.

Wie bei der Probenentnahme und der Analyse, ist auch die Messung des Durchflusses in einer Kläranlage mit Unsicherheiten belegt. Diese Unsicherheiten, die je nach Wetter und Messgerät zwischen 5% und 15% liegen, müssen in der Berechnung der Tagesfrachten berücksichtigt werden. Die berechneten Frachten sind noch keine Schätzung der verbrauchten Mengen an Drogen, da sie sich auf Drogenrückstände beziehen. Sie können aber mit dem Konsum dieser Substanzen direkt in Verbindung gebracht werden. Tagesfrachten können deshalb für Vergleichsstudien (z.B. zwischen verschiedenen Städten) und für langfristige Studien über Zeit benutzt werden.

15.4 Von der Abwassermessung zur konsumierten Menge:

Modellierung des Konsumverhaltens

Nach der Einnahme sind Drogen – aber auch jede andere eingenommene Chemikalie – einem mehr oder weniger umfassenden Prozess der Metabolisierung durch den menschlichen Körper ausgesetzt. Die eingenommenen Substanzen werden am Ende dieses Prozesses hauptsächlich über Urin oder Fäkalien wieder aus dem Körper ausgeschieden. Für die Erstellung eines Modells des Drogenkonsums in der an eine Kläranlage angeschlossen

Bevölkerung, ist die Kenntnis des Verhältnisses zwischen der konsumierten Dosis einer Substanz und der in den Ausscheidungen enthaltenen Drogenrückstände zentral. Dieses Verhältnis bildet die Grundlage für die Modellierung des Konsumverhaltens anhand von Abwasseranalysen.

Pharmakodynamische Studien, in denen Personen eine bekannte Dosis einer Substanz einnehmen und die danach ausgeschiedenen Mengen an Drogenrückständen zu unterschiedlichen Zeitpunkten (nach wenige Minuten, Stunden oder mehreren Tagen) gemessen wurden, liefern Erkenntnisse über dieses Verhältnis. Der größte Teil solcher Studien hat sich auf Drogenrückstände im Urin konzentriert. Es gibt jedoch Hinweise, dass für manche Substanzen der Beitrag von Ausscheidung durch Fäkalien erheblich ist¹.

Basierend auf den im Abwasser gemessenen Frachten von Drogenrückständen kann unter Hinzunahme des Verhältnisses zwischen konsumierter und ausgeschiedener Dosis die Gesamtmenge der innerhalb des Probeentnahmezeitraums (üblicherweise 24 Stunden) von der an die Kläranlage angeschlossenen Bevölkerung konsumierten Substanz errechnet werden. Diese Methode wird als Rückrechnung (vom Englischen *back-calculations*) bezeichnet. Die Formel 5 dient zur Berechnung der konsumierten Kokainmenge anhand der gemessenen Tagesfracht des Kokainmetaboliten Benzoylecgonin:

$$COC [g/Tag] = BE[g/Tag] * \frac{1.04}{30.6\%} \quad \text{Formel 4}$$

In der Formel 5 ist *COC* die ursprüngliche Menge an verbrauchtem Kokain und *BE* die im Abwasser gemessene Tagesfracht an Benzoylecgonin, 30.6% ist der Anteil an eingenommenem Kokain, der als Benzoylecgonin im Urin ausgeschieden wird (35) (z.B. für 1 Gramm verbrauchtes Kokain werden davon etwa 30.6% als Benzoylecgonin ausgeschieden) und 1.04 ist das Verhältnis zwischen dem Molekulargewicht von Kokain (303.35 Gramm pro Mol) und jenem von Benzoylecgonin (289.33 Gramm pro Mol).

Wenn anstatt eines Metaboliten die Frachten einer unveränderten Substanz in die Formel eingesetzt werden, entfällt der letzte Faktor. Wenn möglich sollten jedoch Metabolit-Frachten als Berechnungsgrundlage benutzt werden. Dies, weil sich die ursprüngliche Substanz auch aus Gründen, die nicht mit dem Konsum in Verbindung stehen im Abwasser befinden kann (z.B. Entsorgung von Drogen in Toiletten). Für Drogen, die keine bekannten oder einfach messbaren Metaboliten haben (z.B. Amphetamin und Methamphetamin) oder für die noch keine pharmakodynamische Studien vorliegen (z.B. neue psychoaktive Substanzen) müssen aber die Substanzen selbst gemessen werden. Die damit verbundenen Unsicherheiten sollten bei der Interpretation der Daten in Betracht gezogen werden.

Rückrechnungen können aus zwei Gründen problematisch sein. Erstens stammen die Erkenntnisse über das Verhältnis von konsumierter Substanz und ausgeschiedener Substanz

¹ Zum Beispiel für Cannabis, wird bis zu 65% des verbrauchten THC-Dosis (Hauptwirkstoff von Cannabis) durch Fäkalien ausgeschieden (47). Gleichermassen wird vermutet, dass auch ein Teil der Ausscheidungen von Heroin durch Fäkalien stattfinden kann (35).

bzw. Metabolit zum Teil aus fragwürdigen Untersuchungen (z.B. überholte Methodologie, geringe Anzahl Teilnehmer, Einnahmemethode nicht dem üblichen Konsumverhalten entsprechend, chemische Form der verabreichten Substanz nicht der üblichen chemischen Form der Substanz auf dem illegalen Markt entsprechend). Zweitens existieren zurzeit noch zu wenige Daten über die Ausscheidung von Drogenrückständen auf anderen Wegen als im Urin (hauptsächlich Fäkalien). Dies fällt vor allem für Substanzen ins Gewicht, von denen erwartet werden kann, dass ein erheblicher Teil der Drogenrückstände nicht im Urin ausgeschieden wird. Wenn der Teil dieser Rückstände durch Fäkalien ins Abwasser gelangt und sich zwischen Betätigung der Spülung und der Probeentnahme in diesem löst, kann dies zu einer erheblichen Überschätzung des Verbrauchs führen, wenn ausschliesslich Daten zur Ausscheidung im Urin benutzt werden. Dies könnte erklären, weshalb es zurzeit nicht möglich ist, konsistente Resultate für 6-monoacetylmorphin (Metabolit der ausschliesslich durch den Konsum von Heroin entsteht) (265) und 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH, Hauptmetabolit von Cannabis) zu berechnen.

Weitere Faktoren, die bei Rückrechnungen betrachtet werden müssen, sind eine mögliche Adsorption der Metaboliten an Schwebestoffe (hauptsächlich für hydrophobische Substanzen) – da diese meistens nicht analysiert werden – und Abbau der Drogenrückstände im Abwasser vor der Probeentnahme (z.B. durch spontane Hydrolyse oder mikrobiologische Aktivität). Allerdings wurden verschiedene Studien durchgeführt, die diese Phänomene untersucht haben (86,112–115,121,123,146,166,284) und es teilweise erlauben, Korrekturfaktoren in die Berechnungsmodelle einzuschließen. Neben all diesen Unsicherheitsfaktoren, muss zudem davon ausgegangen werden, dass Abwasserverluste durch lecke Abwasserleitungen und die Architektur des Kanalisationssystems ebenfalls einen Einfluss auf die Schätzungen haben. Dies betrifft insbesondere Vergleichsstudien zwischen unterschiedlichen Städten.

15.4.1.1 Zusammenfassung der wichtigsten Unsicherheitsfaktoren

Die obigen Ausführungen verdeutlichen, dass die im Abwasser gemessenen Frachten eines Drogenmetaboliten und die aus diesen Messungen berechneten konsumierten Mengen einer Substanz einer Reihe von Unsicherheiten ausgesetzt sind. Zusammengefasst hat die Fachliteratur folgende Unsicherheitsfaktoren identifiziert (122,146).

- Probenahme
- Durchflussmessungen
- Messmethodik/Analytik
- Pharmakodynamik und Ausscheidungswege
- Abbau der Substanz vor Probenahme / Verlust im Kanalisationssystem
- Adsorption an Schwebstoffe
- Architektur des Kanalisationssystems (Vergleichsstudien)

Wichtig ist zu beachten, dass diese Faktoren zwar zur Unsicherheit der Schätzungen beitragen, ihr Einfluss auf Langzeitstudien aber weniger erheblich ist. Da Langzeitstudien im

selben Einzugsgebiet durchgeführt werden kann deshalb der Beitrag vieler dieser Unsicherheitsfaktoren über die Zeit als konstant angenommen werden. Somit können Tendenzen in Abwassermessungen in einen Einzugsgebiet mit grosser Wahrscheinlichkeit auf Veränderungen im Konsumverhalten oder in der Qualität des Produktes am Markt zurückgeführt werden.

15.5 Der Kokainmarkt in Lausanne: Potential und Grenzen von Abwasserdaten

Die praktischen Auswirkungen dieser Unsicherheiten auf die Analyse und Interpretation von Daten aus Abwasseranalysen lassen sich aus den Ausführungen im ersten Teil dieses Artikels nicht direkt ableiten. In diesem Teil wird anhand eines praktischen Beispiels aufgezeigt, wie bei der Interpretation solcher Messwerte vorgegangen werden kann und welche Rückschlüsse trotz aller Unsicherheiten auf einen Drogenmarkt gezogen werden können. Dazu wird ein bisher nicht veröffentlichter Datensatz von 43 Analysen des Abwassers der Stadt Lausanne² auf den Kokainmetaboliten Benzoylecgonin verwendet. Der Datensatz ist interessant, weil er eine Reihe von Proben umfasst, die über den Zeitraum von etwas mehr als einem Jahr in zweiwöchentlichem Abstand in der Regel an einem Samstag und dem vorangehenden oder darauffolgenden Dienstag aus dem Abwasser derselben Stadt gezogen wurden. Der Datensatz gibt also Auskunft über mögliche Fluktuationen des Konsumverhaltens im Verlauf eines Jahrs und auch im Verlauf der jeweiligen Woche. Die Analyse in diesem Abschnitt umfasst einen deskriptiven Teil, in dem Muster im Datensatz identifiziert und beschrieben werden und einen interpretativen Teil, in dem versucht wird, aus diesen Mustern Erkenntnisse über den Kokainmarkt in der Stadt Lausanne abzuleiten.

² Das Einzugsgebiet der Kläranlage in Lausanne, welches die Stadt Lausanne und 15 umliegende Gemeinden umfasst, hat eine Bevölkerung von etwa 226 000 Einwohnern.

15.5.1 Deskriptive Analyse

Ein Überblick über die im Datensatz enthaltenen 43 Messungen zeigt zwei Auffälligkeiten (siehe Abb. 57 und Tab. 47).

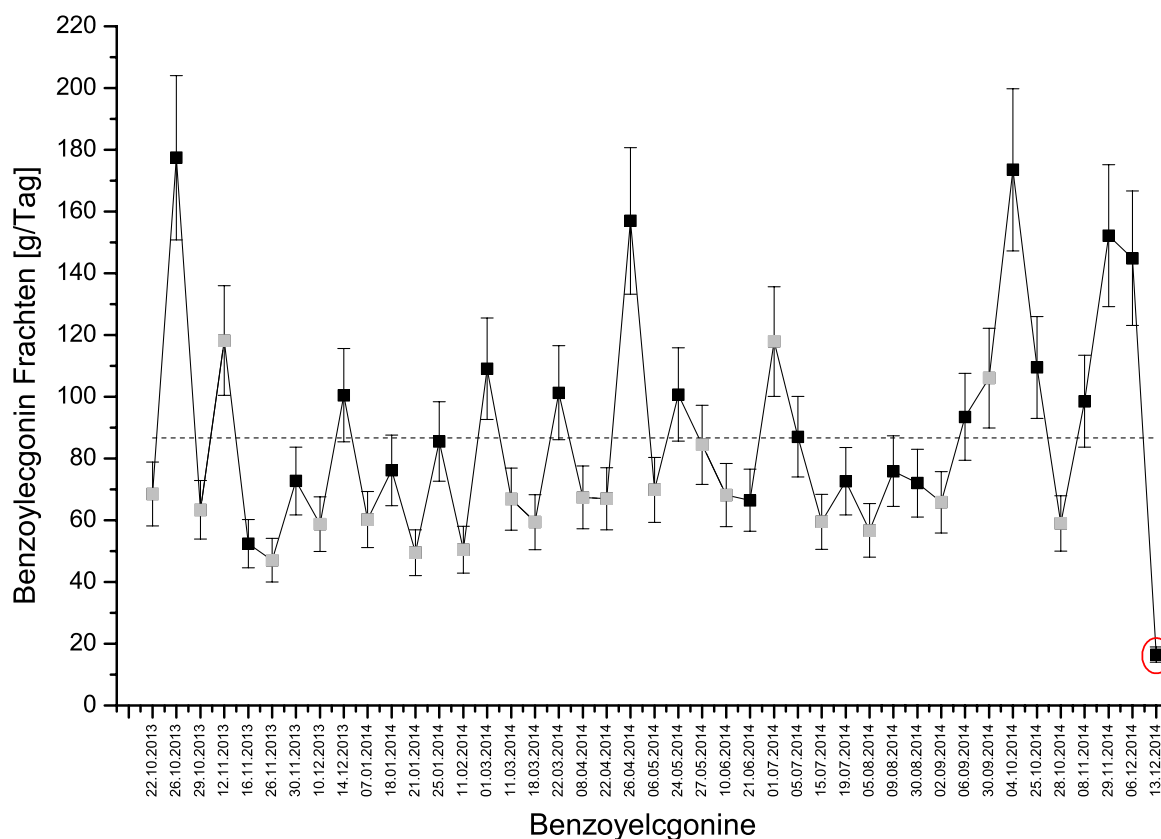


Abb. 57: Gemessene Benzoyllecgonin Frachten in Lausanne zwischen Oktober 2013 und Dezember 2014. In Grau: Messungen von Dienstag auf Mittwoch (12 Uhr – 12 Uhr); In Schwarz: Messungen von Samstag auf Sonntag (12 Uhr – 12 Uhr). Beim rot umkreisten Messwert handelt es sich um einen Ausreisser.

Tab. 47: Gemessene Benzoylecgonin-Frachten und entsprechender Kokainverbrauch (ohne Ausreiser) in Gramm reiner Substanz pro Tag. Standardabweichung des Mittelwert (Standardfehler) = Standardabweichung der gesamten Daten geteilt durch die Wurzel der Anzahl Messungen (ohne Ausreiser; $\sqrt{42}$).

		<i>Benzoylecgonin Frachten [g/Tag]</i>	<i>Kokain Konsum [g/Tag]</i>
<i>Alle Daten</i>	Mittelwert	86.7	301.2
	Standardabweichung	33.8	117.5
	Standardabweichung des Mittelwerts	14.0	60.9
	Min	47.0	163.4
	Max	177.4	616.1
<i>Dienstag – Mittwoch</i>	Mittelwert	69.7	242.1
	Standardabweichung	20.4	70.7
	Standardabweichung des Mittelwerts	10.9	47.5
	Min	47.0	163.4
	Max	118.2	410.3
<i>Samstag – Sonntag</i>	Mittelwert	100.3	348.2
	Standardabweichung	36.4	126.4
	Standardabweichung des Mittelwerts	16.5	71.8
	Min	52.4	182.0
	Max	177.4	616.1

Erstens lässt sich ein eindeutiger Ausreißer feststellen. Der Messwert der Proben vom 13. Dezember 2014 ist um das circa Fünffache geringer als der Durchschnitt aller 43 Messwerte im Datensatz. Es gibt gute Gründe davon auszugehen, dass dieser Messwert für den Kokainkonsum in Lausanne nicht repräsentativ ist. An diesem Tag wurden außergewöhnlich niedrige Durchflüsse gemessen, die auf ein Problem mit dem Durchflusssensor hinweisen. Deshalb wird der Ausreiser in der restlichen deskriptiven Analyse nicht beachtet.

Zweitens zeigt sich auch ohne die Berücksichtigung des Extremwerts, dass die Unterschiede zwischen den einzelnen Messwerten erheblich sind. Der höchste Messwert von 177.4 Gramm Benzoylecgonin pro Tag (Samstag, 26. Oktober 2013) ist fast viermal höher als der tiefste gemessene Wert von 47.0 Gramm Benzoylecgonin pro Tag (Dienstag, 26. November 2013). Insgesamt kann aber gesagt werden, dass 32 der 42 als verlässlich betrachteten Messwerte innerhalb der Spanne von +/- 1 Standardabweichung (33.8 Gramm Benzoylecgonin pro Tag) vom Mittelwert liegen (52.9 Gramm Benzoylecgonin pro Tag bis 120.6 Gramm Benzoylecgonin pro Tag). Dies entspricht grundsätzlich einer Streuung, wie sie bei Annahme einer Normalverteilung der Grundgesamtheit erwartet werden kann.

Im Jahresverlauf lassen sich keine systematischen Fluktuationen der gemessenen Menge Benzoylecgonin feststellen (siehe Abb. 57). So gibt es zum Beispiel keine Hinweise darauf, dass in der wärmeren oder kälteren Jahreszeit mehr oder weniger konsumiert wird. Auffällig ist hingegen, dass 17 der 20 Messwerte oberhalb des Median an Samstagen gemessen wurden. Ebenfalls fällt auf, dass elf der 20 Werte oberhalb des Median an Tagen gemessen wurden, an denen seit der letzten Lohnzahlung eine Woche oder weniger vergangen war

(typischerweise werden die Löhne in der Schweiz ab dem 25. des jeweiligen Monats ausbezahlt). Alle neun Samstage innerhalb einer Woche nach der letzten Lohnzahlung, für die Messwerte vorliegen, liegen oberhalb des Median. Statistische Analysen bestätigen, dass sich der Mittelwert von diesen und jener aller anderer Samstage (jene elf Samstage die nicht innerhalb eine Woche nach der Lohnzahlung gemessen wurden) unterscheiden (Wilcoxon-Mann-Whitney-Test p -Wert = 0.08, α = 0.05). Unterhalb des Median befinden sich 17 Dienstage, von denen 15 nicht in der Woche nach der letzten Lohnzahlung zu liegen kamen. Auch in Bezug auf die an Dienstagen gemessenen Werten bestätigte die statistische Analyse, dass sich der Mittelwert von Dienstagen kurz nach Lohnzahlung von jenen aller anderen Dienstage unterscheidet (Wilcoxon-Mann-Whitney-Test p -Wert = 0.46, α = 0.05).

Entsprechend der erheblichen Unterschiede zwischen den an Samstagen und den an Dienstagen gemessenen Werten scheint es gerechtfertigt, die beiden Wochentage zu gruppieren und separate Mittelwerte und Standardabweichungen zu berechnen. An Samstagen liegt der Mittelwert 100.3 Gramm Benzoyllecgonin pro Tag an Dienstagen bei 69.7 Gramm Benzoyllecgonin pro Tag. Die Standardabweichung der Stichprobe der Samstag-Gruppe beträgt 36.4 Gramm Benzoyllecgonin pro Tag, jene für die Dienstag-Gruppe 20.4 Gramm Benzoyllecgonin pro Tag.

Anhand der oben beschriebenen Formel lässt sich aus den gemessenen Benzoyllecgonin-Werten auch eine Menge an konsumiertem Kokain (reine Substanz) pro Tag, an dem eine Messung stattgefunden hat, berechnen. Dies ist zulässig, weil die Probeentnahme so vorgenommen wurde, dass davon ausgegangen werden kann, dass der gemessene Benzoyllecgonin-Wert wahrscheinlich repräsentativ ist, für die an diesem Tag im Lausanner Abwasser vorhandene Menge an Benzoyllecgonin. Ausserdem wurden die beschriebenen Unsicherheiten in den Schätzungen berücksichtigt und sind in den berechneten Standardabweichungen eingeschlossen. Die konsumierte Menge reines Kokain schwankte an den Messtagen gemäß dieser Schätzung zwischen 163.4 ± 30.8 g (26. November 2013) und 616.1 ± 115.7 g (26. Oktober 2013).

Der Mittelwert aller Messungen (ohne Ausreiser) beträgt 301.2g konsumiertes Kokain pro Tag, die Standardabweichung vom Mittelwert über die gesamte Stichprobe (ohne Ausreiser) beträgt 60.9g Kokain. Werden die Mittelwerte und die entsprechende Standardabweichung für die beiden Wochentage separat berechnet, ist sie an Samstagen deutlich höher (Mittelwert: 348.2 ± 71.8 g) als an Dienstagen (Mittelwert: 242.1 ± 47.5 g).

15.5.2 Interpretation

Welche Interpretation lassen diese Daten zu? Eine erste Feststellung bezieht sich auf die Fluktuation der gemessenen Menge an Benzoyllecgonin im Abwasser. Wenn die im Abwasser gemessenen Frachten tatsächlich die in der Stadt konsumierte Menge Kokain widerspiegeln, schwankt diese beträchtlich. Ohne Hinzunahme weiterer Informationen scheint jedoch nicht offensichtlich, warum diese Schwankungen auftauchen. Offensichtlich spielen Wochentage eine zentrale Rolle für die Unterschiede innerhalb der gesamten Stichprobe, aber auch wenn

alle Samstage und alle Dienstage gruppiert betrachtet werden, ist die Varianz innerhalb dieser Gruppen von Datenpunkten immer noch erheblich.

Während es offensichtlich erscheint, dass an Wochenenden in Lausanne mehr Kokain konsumiert wird als an anderen Wochentagen, lassen die vorliegenden Daten allein jedoch offen, warum dies der Fall ist. Ob der erhebliche Unterschied der Mittelwerte und Standardabweichungen der beiden Gruppen vom Datenpunkt durch die Anwesenheit von mehr Konsumierenden in der Stadt, einem erhöhten Konsum der in der Stadt wohnhaften regelmässig Konsumierenden, einem häufigeren Auftreten von Gelegenheitskonsum an Wochenenden (durch in der Stadt wohn- und/oder nichtwohnhafte Personen) oder durch eine Kombination mehrerer oder aller dieser Faktoren ausgeht, lässt sich ausschliesslich aufgrund von Benzoylcegonin-Werten im Abwasser nicht feststellen.

Dasselbe gilt auch für die beobachteten höheren Messwerte im zeitlichen Umfeld von Lohnzahlungen. Selbst wenn davon ausgegangen wird, dass diese Beobachtung direkt mit der Verfügbarkeit von finanziellen Mitteln im Zusammenhang steht, ist nicht klar, ob regelmässig Konsumierende, Gelegenheitskonsumierende oder temporär in der Stadt anwesende Personen oder alle drei Personengruppen gleichzeitig für die gesteigerte Menge an Benzoylcegonin im Abwasser bzw. den erhöhten Kokainkonsum verantwortlich sind³.

Das kleinere Verhältnis zwischen Mittelwert und Standardabweichung aller am Dienstag gezogenen Proben suggeriert zwar, dass zumindest ein Teil der Varianz von sich temporär in der Stadt befindlichen Personen generiert wird. Ohne zusätzliche Informationen über die Anzahl an Personen, die sich an Wochenenden nach Lausanne begeben und über ein mögliches unterschiedliches Konsumverhalten von in der Stadt ansässigen Konsumierenden, bleibt diese Feststellung aber Spekulation.

Angesichts der im Datensatz vorhandenen Varianz und den diskutierten Schwierigkeiten bezüglich ihrer Erklärung ohne die Hinzunahme weiterer Daten, stellt sich die Frage, ob basierend auf den vorliegenden Daten eine Schätzung der in Lausanne jährlich konsumierten Menge Kokain vertretbar ist. Unabhängig von der Frage, ob eine Stichprobe von 41 Tagen bzw. 21 Samstagen und 20 Dienstagen repräsentativ für ein ganzes Jahr sein kann, bietet der vorliegende Datensatz mindestens zwei Möglichkeiten, die Menge an konsumiertem Kokain in Lausanne zu berechnen.

Erstens kann der Mittelwert der gesamten Stichprobe (291g) als Schätzung für den Tagesverbrauch angenommen werden. Daraus ergäbe sich ein geschätzter Kokainkonsum von 106.2 kg ($291\text{g} \cdot 365$) pro Jahr. In diesem Modell muss jedoch angesichts der Standardabweichung in der Stichprobe eher davon ausgegangen werden, dass der Kokainbedarf in Lausanne mit einer gewissen Wahrscheinlichkeit zwischen 87.7 kg ($(301.2\text{g} - 60.9\text{g}) \cdot 365$) und 132.2 kg ($(301.2\text{g} + 60.9\text{g}) \cdot 365$) liegt.

³ Über einen Unterschied zwischen Messwerte im Umfeld der Lohnzahlung und „normale“ Tage wurde in der Fachliteratur bisher noch nicht berichtet. Um diesen Befund zu bestätigen, wären zusätzliche Analysen notwendig.

Zweitens kann aufgrund der Messwerte davon ausgegangen werden, dass an Samstagen in Lausanne besonders viel Kokain konsumiert wird und die Messwerte der Dienstage repräsentativ für die restlichen Wochentage sind. So berechnet ergäbe sich an Samstagen ein Konsum von 18 kg ($52 \cdot 348.2$ g; Spanne: 14.4 kg bis 21.8 kg). Wenn man davon ausgeht, dass der Konsum an Freitagen ähnlich ist wie an Samstagen, würde der Kokainverbrauch über Wochenenden (Freitage + Samstage) bei 36.2 kg pro Jahr liegen (Spanne: 28.7 kg bis 43.7 kg). An Wochentagen würden 63.2 kg ($261 \cdot 242.1$ g; Spanne: 50.8 kg bis 75.6 kg) konsumiert. Die Schätzung des jährlichen Konsums gemäss dieser Berechnungsart würde sich auf 99.4 kg reines Kokain belaufen (Spanne: 79.5 kg bis 119.3 kg).

Weil die für die Berechnungen benutzten Parameter mit Unsicherheiten belegt sind, ist es schwierig, eine präzisere Schätzung des Kokainkonsums in Lausanne abzugeben. Da es aber für andere Substanzen (Methadon und Heroin) möglich war, anhand von Abwassermessungen getroffene Schätzungen durch epidemiologische Daten zumindest für die Stadt Lausanne zu validieren (265), kann davon ausgegangen werden, dass die errechneten Werte für den Kokainverbrauch in Lausanne und seinem Einzugsgebiet eine realistische Größenordnung wiedergeben. Aber auch wenn man davon ausgeht, dass diese Schätzung eine gewisse Aussagekraft hat, lassen die vorliegenden Daten offen, wie viele Personen diese Menge Kokain konsumieren. Darüber lässt sich ebenfalls nur unter Annahme weiterer Faktoren spekulieren.

15.6 Schlussfolgerung: Der polizeiliche Nutzen von Abwasserdaten

Können aus den Daten aus der Abwasseranalyse für die Polizei nützliche Informationen gewonnen werden, die anderweitig nicht verfügbar wären? Aus operativer Perspektive dürften die im vorliegenden Datensatz vorhandenen Informationen nur bedingt von Nutzen sein. Zwar scheinen die gemessenen Benzoyllecgonin-Frachten zu bestätigen, was nicht nur in polizeilichen Kreisen bereits vermutet wurde: an Wochenenden dürfte der Kokainkonsum zumindest in städtischen Gebieten höher sein. Darüber hinaus lässt sich aber aus den Daten wenig ableiten, was direkt auf operative Tätigkeiten der Polizei anwendbar ist.

In strategischer Hinsicht scheint der Datensatz zu den Benzoyllecgonin-Frachten im Lausanner Abwasser und die daraus resultierenden Berechnungen bezüglich der konsumierten Menge Kokain Informationen zu erschließen, die ohne den Datensatz nicht ohne weiteres bekannt wären. Die Information, dass in Lausanne jährlich mit einiger Wahrscheinlichkeit zwischen 79.5 kg und 132.2 kg reines Kokain konsumiert werden mag zwar eher vage erscheinen. Dadurch kann aber die Einschätzung einer Sicherstellung als Anteil der gesamten in einer Stadt konsumierten Menge vereinfacht werden, auch wenn eine eindeutige Angabe aufgrund der statistischen Unsicherheit von $\pm 20\%$ nicht möglich ist.

In ähnlicher Weise kann dieser Wert verwendet werden, um Schätzungen aus anderen Datenquellen besser einzuordnen. Solche Informationen sind wichtig, um ein besseres Verständnis für den Kontext von Sicherstellungen und Ermittlungsergebnissen zu entwickeln. Die Einschätzung, zum Beispiel, ob eine Gruppierung ein Prozent oder zehn Prozent des

Kokainmarkts in einer Stadt versorgt, kann für die Allokation von Ermittlungsressourcen von erheblicher Wichtigkeit sein, auch wenn der Marktanteil dieser Gruppierung nicht noch genauer beziffert werden kann. Dasselbe gilt für die Einschätzung von Umsätzen oder Gewinnen aus dem Drogenhandel („Ist ein Straßenwert einer Sicherstellung von 800 000 Fr. viel oder wenig?“) die im Hinblick auf den Gesamtkontext in dem sie stattfinden besser interpretiert werden können, als wenn sie ohne Kontext diskutiert werden.

Der volle Nutzen der Abwasseranalytik dürfte vor allem dann zum Tragen kommen, wenn genügend lange Zeitreihen zur Verfügung stehen. Für Vergleiche über die Zeit hinweg spielen die diskutierten Messunsicherheiten eine deutlich kleinere Rolle. Dies ermöglicht die Identifikation von Tendenzen im Konsumverhalten. Denn unabhängig davon, welcher Menge Kokain ein bestimmter Wert von Benzoyllecgonin im Abwasser entspricht, kann davon ausgegangen werden, dass eine Ab- oder Zunahme des Benzoyllecgonin-Wertes auch einer Zu- oder Abnahme des konsumierten Kokains entspricht.

Die Abwasseranalyse kann zudem nützliche Informationen in Bezug auf geographische Unterschiede im Drogenkonsum liefern. Die Methode ermöglicht es – zumindest in Regionen, in denen Kläranlagen weit verbreitet sind – die Drogenrückstände im Abwasser verschiedener Städte gleichzeitig zu analysieren und Informationen über regionale Unterschiede zu gewinnen.

Alle Fragen kann aber auch die Abwasseranalyse nicht beantworten. Die Messung einer direkten Auswirkung von Sicherstellungen auf die Höhe der Benzoyllecgonin-Frachten scheint angesichts der im Datensatz beobachtbaren Schwankungen eher problematisch. Der Kokainmarkt ist wahrscheinlich flexibel genug, um relativ schnell auf neue Belieferungsquellen zurückgreifen zu können. Der direkte Einfluss auch einer großen Sicherstellung auf die Benzoyllecgonin-Frachten im Abwasser dürfte deshalb – wenn überhaupt – nur kurzfristig messbar sein. Um solche Fragen zu beantworten, müssten Abwassermessungen enger mit polizeilichen Massnahmen koordiniert und in die polizeiliche Planung aufgenommen werden. Gleichzeitig wird es aber weiterhin nicht möglich sein aus diesen Messwerten direkt abzuleiten wie viele Personen eine Substanz konsumieren. Dies benötigt die Berücksichtigung von Daten, die nur aus Bevölkerungsbefragungen (Tages- oder Monatskonsum von regelmässigen und Gelegenheitskonsumierenden) und Sicherstellungen (Reinheit von Produkten die auf dem Markt sind) hervorgehen. Gemeinsam mit andere Datenquellen über Drogenkonsum trägt aber die Abwasseranalyse bereits heute dazu bei, sich ein klareres Bild der Situation betreffend des illegalen Marktes zu verschaffen (242,243,265).

In diesem Sinne ist die Abwasseranalytik ein zusätzliches Instrument zum Verständnis des gesellschaftlichen Phänomens Drogen. Ihre Daten sollten immer im Verbund mit anderen Daten sinnvoll interpretiert werden. Um das Potential der Abwasseranalytik für ein besseres Verständnis von Drogenmärkten zu nutzen, braucht es aber eine kontinuierliche Sammlung von Daten. Ohne einen möglichst vollständigen Satz von Abwasserproben sind weder eine sinnvolle Einordnung in andere Daten noch die Identifikation von Mustern über die Zeit hinweg möglich.

XVI References

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