Zeitschrift: Kriminologie / Schweizerische Arbeitsgruppe für Kriminologie SAK = Criminologie / Groupe Suisse de Criminologie GSC = Criminologia / Gruppo Svizzero di Criminologia GSC

Herausgeber: Schweizerische Arbeitsgruppe für Kriminologie

Band: 34 (2017)

Artikel: Illicit drugs in wastewater : a new perspective for criminological evaluation

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DOI: https://doi.org/10.5169/seals-1051466

Illicit drugs in wastewater – a new perspective for criminological evaluation

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Summary

Wastewater-based epidemiology (WBE) uses wastewater as a source of information about the lifestyle and health status of a given community. Through the analysis of relevant markers, in particular residues of illicit drugs, indications about the type and amount of substances being used can be derived. Although a recent discipline, significant developments have been made in the last decade and WBE has been applied in numerous cities across the world. Recent studies have tackled the possibility of combining results derived from WBE with data derived from conventional indicators, such as general population surveys, treatment statistics (e.g., syringe distribution programs) and law enforcement data (e.g., seizures, informants). These studies have shown that WBE not only provides quantitative, temporal and spatial information about drug use, but that if it is evaluated in perspective of other types of indicators, it can help better understand and monitor illicit drug use at the local, national and international scale.

1. Introduction

Monitoring the consumption of illicit drugs and, to a broader extent, the lifestyle and health status of entire populations via wastewater analysis is a novel approach, referred to as wastewater-based epidemiology (WBE). The approach was first formalized in 2001 by Daughton[1], who suggested that community-wide information, in particular about illicit drug use, could be obtained by analyzing specific urinary markers in wastewater samples. Concretely, however, the technique was implemented for the first time only in 2005 to monitor consumption of illicit drugs in the city of Milan.[2] Ever since, a great number of scientists have focused their research efforts on refining the scientific base of this discipline and extending its application to other fields.

1.1 Principles

Wastewater-based epidemiology relies on the principle that any exogenous substance (xenobiotic) entering the human body will, after a more or less extensive modification process (i.e., metabolism), be eliminated (excreted). The substance will be excreted either unchanged (parent compound) or as a metabolite (i.e., slightly modified version of the parent compound). Excretion will take place mainly via urine and faeces, but, though to a substantially lower extent, also through sweat and saliva.[3] These markers will, through sanitary appliances (e.g., toilets, showers), enter the sewer system and eventually reach a wastewater treatment plant (WWTP). Following the same principle of toxicological or clinical urinalysis, the occurrence and levels of these substances in wastewater can, through their detection and suitable analysis, provide clues about the consumption of (illicit) drugs, exposure to pollutants or the health status of the studied community.

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1 DAUGHTON, C. G. Illicit Drugs in Municipal Sewage: Proposed New Non-intrusive Tool to Heighten Public Awareness of Societal Use of Illicit/Abused Drugs and Their Potential for Ecological Consequences, in: C.G. Daughton and T.L. Jones-Lepp (Eds.), Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues, Symposium Series 791, American Chemical Society: Washington DC, 2001, pp. 348-364.

2 ZUCCATO E. ET AL., Cocaine in Surface Waters: A New Evidence-Based Tool to Monitor Community Drug Abuse, *Environmental Health*, 2005.

3 JENKINS, A. J. Pharmacokinetics: Drug Absorption, Distribution, and Elimination, in: Drug Abuse Handbook, CRC Press, Boca Raton FL, 1998, p. 151.

1.2 Wastewater sampling

The objective of WBE is to gather information at community or population level; therefore samples are generally collected at the influent of a wastewater treatment plant. The reason for this is that samples should contain raw wastewater, e.g. wastewater that has not undergone any treatment, to limit modification in marker levels. How samples are collected plays a crucial role in WBE as it will have a major impact on the validity and the informative value of the obtained results, and thus affect their interpretation.[4] Generally, wastewater sampling is carried out using an autosampler, basically a pump whose operation frequency (in minutes) and sampled volume (in milliliters) can be adjusted. Wastewater samples are then collected in bottles that are stored in a refrigerator connected to the pumping system. Treatment plants are generally equipped with these devices since they have to collect wastewater samples to guarantee compliance with existing regulations, yet smaller plants will likely only have autosamplers installed to collect samples from effluents.[5]

The period during which samples are collected and the sampling rates must be carefully adjusted in accordance with the question to be answered. In other words, the information that can be obtained from the results is directly and highly dependent on the number, the composition and the quality of the samples. The sampling strategy is thus a key parameter that has to be thought about in priority. In WBE studies, sampling is commonly done over 24 hours, so as to obtain a sample representative of the daily marker load (i.e., amounts of substance, in milligrams or grams, having reached the WWTP during one day). In WBE monitoring campaigns, analyzed samples have often been collected on consecutive days, as it is the case for the international monitoring study[6], where samples were collected over one week. However, alternative approaches can be contemplated depending on the type of information requited.

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4 ORT, C. ET AL., Sampling for PPCPs in Wastewater Systems: Comparison of Different Sampling Modes and Optimization Strategies, *Env. Sci. Technology*, 2010, 44(16):6289-96; ORT C. ET AL., Sampling for Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs in Wastewater Systems: Are Your Conclusions Valid? A Critical Review, ibid, 6024-35.

5 ORT C. ET AL., Challenges of Surveying Wastewater Drug Loads of Small Populations and Generalizable Aspects on Optimizing Monitoring Design, *Addiction*, 2014, 109(3): 472-81.

6 SCORE, "Sewage Analysis CORe Group Europe."

1.3 Analysis of drug residues in wastewater

After collection, raw wastewater samples can be either processed and analyzed immediately or frozen (i.e., -20°C) for later analysis. Before analysis, however, the samples have to be processed in order to extract and pre-concentrate the analytes. This is generally achieved using a technique called *solid-phase extraction* (SPE). This technique allows to concentrate the analytes (e.g., by a factor to up to 1000) and to simultaneously reduce the interference of the matrix (i.e., wastewater and its content). Solid-phase extraction, which combines clean-up and preconcentration, is among the most common techniques routinely implemented in clinical and toxicological analysis of biological matrices (e.g., blood and urine).

After sample preparation, analyses are generally carried out using liquid chromatography (LC) coupled to tandem (MS/MS) or high-resolution mass spectrometry (HRMS). These highly sophisticated analytical instruments allow to selectively and sensitively detect and quantify trace amounts (i.e., nanograms per milliliter or lower) of analytes in various types of matrices (e.g., environmental, biological, pharmaceutical, foods).[7]

1.4 Relevant illicit drug markers

Illicit drugs have been the focus of most WBE applications. Cannabis, cocaine, amphetamine, methamphetamine, ecstasy (or MDMA) and opiates (e.g. heroin, morphine, methadone, oxycodone, fentanyl) are among the most commonly monitored substances, although some new psychoactive substances (NPS) have recently also been studied.[8]

As briefly mentioned previously, after consumption, illicit drugs are excreted unchanged or in the form of metabolites. These constitute the markers which are generally looked for in WBE studies. The amount of parent compound or metabolite which will be excreted, for example in

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7 BIJLSMA, L. ET AL., Improvements in Analytical Methodology for the Determination of Frequently Consumed Illicit Drugs in Urban Wastewater, *Analytical and Bioanalytical Chemistry*, 2014, 406(17); HERNÁNDEZ F. ET AL., Investigation of Pharmaceuticals and Illicit Drugs in Waters by Liquid Chromatography-High-Resolution Mass Spectrometry, *Trends in Analytical Chemistry*, 2014, 63, 140-157.

8 KINYUA J. ET AL., Sewage-Based Epidemiology in Monitoring the Use of New Psychoactive Substances: Validation and Application of an Analytical Method Using LC-MS/MS, *Drug Testing and Analysis*, 2015, 7(9): 812-818.

urine or in faeces, will in principle depend on the amount of substance initially consumed. The ratio between the initial dose and the excreted amount is generally referred to as the *excretion rate* (expressed in percentage). Yet, individual factors such as sex, age, weight and health status, or the administration route (e.g., fumigation, intravenous injection, oral and nasal insufflation) can influence these ratios.[9] A list of the substances analyzed in WBE studies is reported below.

Table 1: List of illicit drugs and their metabolites analyzed in wastewater

Illicit drug	**Wastewater marker**	**Note**
Cannabis	THC-COOH	Principal marker measured in wastewater. However, there is limited data about its excretion, in particular via faeces.[10] Furthermore, its analysis poses some peculiar challenges due to its potential adsorption onto plastic.[11]
	OH-THC	Has been analysed in a limited number of studies because less stable than THC-COOH.[12]
Cocaine	Cocaine	Is regularly analysed in wastewater samples, although benzoylecgonine is preferred because the occurrence of cocaine in wastewater could be due to reasons other than consumption (i.e., direct disposal).

9 BASELT, R. C. AND CRAVEY R. H., Disposition of Toxic Drugs and Chemicals in Man, 1994; JENKINS, A. J., Pharmacokinetics: Drug Absorption, Distribution, and Elimination, *see note 3*.

10 HUESTIS, M. A., Human Cannabinoid Pharmacokinetics, *Chem. Biodivers.*, 2007, 4(8): 1770-1804.

11 MCCALL, A. K. ET AL., Critical Review on the Stability of Illicit Drugs in Sewers and Wastewater Samples, *Water Res.*, 2016; SENTA, I. ET AL., Assessment of Stability of Drug Biomarkers in Municipal Wastewater as a Factor Influencing the Estimation of Drug Consumption Using Sewage Epidemiology, *Sci. Total Environ.*, 2014.

12 POSTIGO, C., DE ALDA, M. L. AND BARCELÓ, D., Evaluation of Drugs of Abuse Use and Trends in a Prison through Wastewater Analysis, *Environment International*, 2011, 37(1): 4955.

Illicit drug	Wastewater marker	Note
	Benzoylecgonine	Exclusive metabolite of cocaine which has been analyzed in wastewater since the first applications of WBE.[13]
	Cocaethylene	Excreted after co-consumption of cocaine and alcohol, has been measured in wastewater.[14]
Amphetamine	Amphetamine	The parent compound is analyzed as it is the major excretion product and there is no other known exclusive metabolite. However, it is also an excretion product of methamphetamine.[15] Similarly to cocaine, direct disposal cannot be excluded.
Methamphetamine	Methamphetamine	The parent compound is analyzed as it is the major excretion product and, except for amphetamine, there are no other known exclusive metabolites. Similarly to cocaine and amphetamine, direct disposal cannot be excluded.
MDMA (Ecstasy)	MDMA	The parent compound is the major excretion product. However, its excretion rate has been shown to depend on the administered dose.[16]
	HMMA	Exclusive metabolite of MDMA[17], however its use in WBE studies is still limited.[18]

13 ZUCCATO, E. ET AL., Cocaine in Surface Waters: A New Evidence-Based Tool to Monitor Community Drug Abuse, *see note 2*.

14 RODRÍGUEZ-ÁLVAREZ, T. ET AL., Alcohol and Cocaine Co-Consumption in Two European Cities Assessed by Wastewater Analysis, *Sci. Total Environ.*, 2015.

15 BASELT AND CRAVEY R. H., Disposition of Toxic Drugs and Chemicals in Man, *see note 9*.

16 DE LA TORRE, R. ET AL., Non-Linear Pharmacokinetics of MDMA ('ecstasy') in Humans, *Br. J. Clin. Pharmacol.*, 2000, 49(2): 104-109; MUELLER M. ET AL., Simultaneous Liquid Chromatographic-Electrospray Ionization Mass Spectrometric Quantification of 3,4-Methylenedioxymethamphetamine (MDMA, Ecstasy) and Its Metabolites 3,4-Dihydroxymethamphetamine, 4-Hydroxy-3-Methoxymethamphetamine and 3,4-Methylenedioxyamphetamine in Squirrel Monkey and Human Plasma after Acidic Conjugate Cleavage, *Forensic Sci. Int.*, 2009, 184(1-3): 64-68; MUELLER M. ET AL., Direct Comparison of (±) 3,4-Methylenedioxymethamphetamine ('ecstasy') Disposition and Metabolism in Squirrel Monkeys and Humans, *Ther. Drug Monit.*, 2009.

17 MAURER H. H. ET AL., Toxicokinetics and Analytical Toxicology of Amphetamine-Derived Designer Drugs ('Ecstasy'), *Toxicol. Lett.*, 2000.

18 BÉEN, F. ET AL., Population Normalization with Ammonium in Wastewater-Based Epidemiology: Application to Illicit Drug Monitoring, *Sci. Technol.*, 2014, 48(14): 8162-9.

Illicit drug	Wastewater marker	Note
Heroin	6-Monoacetyl-morphine	Exclusive metabolite of heroin which is generally analyzed in wastewater, although not always detected because rapidly transformed to morphine in wastewater. Whilst estimates of consumption based on this marker have been shown to be inconsistent (i.e., limited data about excretion rates), it can be used to monitor heroin consumption over time.[19]
	Morphine	Major excretion product of heroin. Has been analyzed in numerous studies, however its occurrence in wastewater is also due to (legal) consumption of pharmaceuticals containing morphine, codeine, pholcodine, nicomorphine and ethylmorphine.[20] Sales and prescription data of these pharmaceuticals are required before consumption estimates can be calculated.[21] These can be purchased from companies conducting market studies for the pharmaceutical industry.
Methadone	Methadone	Has been analyzed in numerous WBE studies and, when detailed data about its prescription exists (i.e., opioid substitution therapy), it can be used to partially validate results obtained from wastewater analysis.
	EDDP	Has also been analyzed in numerous studies and has been shown to be a more reliable marker than the parent compound to estimate consumption.[22]

19 Béen F. et al., Data Triangulation in the Context of Opioids Monitoring via Wastewater Analyses, *Drug and Alcohol Dependence*, 2015, 151, 203-210.

20 Khan U. and Nicell, J., Refined Sewer Epidemiology Mass Balances and Their Application to Heroin, Cocaine and Ecstasy, *Environment International, 2011, 37(7): 1236-52.*

21 Béen F. et al., Data Triangulation in the Context of Opioids Monitoring via Wastewater Analyses, *see note 19.*

22 Ibid.

Illicit drug	Wastewater marker	Note
New psychoactive substances	Mephedrone Methoxetamine Butylone Ethylone Methylone 4-Methoxy-methamphetamine Benzylpiperazine ...	Various NPS have been analyzed in published[23] and ongoing projects in Switzerland (i.e., mephedrone and methylone have been detected in analyses carried out at the Ecole des Sciences Criminelles) and abroad. Yet, monitoring NPS use via wastewater analysis has some issues, namely because of the ever increasing number of substances which appear in the market and the limited pharmacokinetic data (i.e., metabolism and, in particular, excretion). This makes it difficult to know which markers to target when analyzing wastewater samples. Furthermore, because individual substances are likely consumed by a limited number of users, their concentrations in wastewater are likely very low.

2. From wastewater analysis to epidemiological indicators

The markers presented in the previous section have, or could, be analyzed in wastewater samples collected from WWTP. Whilst measured concentrations provide an indication about the consumption of a specific drug in the investigated population, they do not inform about consumption patterns (i.e., trends) nor can they be used to compare results between different sampling locations. Thus, concentrations are generally multiplied by wastewater flows (measured by the WWTP personnel) to obtain absolute loads (i.e., total amount of biomarker having reached the WWTP),

23 CASTIGLIONI S. ET AL., Wastewater Analysis to Monitor Spatial and Temporal Patterns of Use of Two Synthetic Recreational Drugs, Ketamine and Mephedrone, in Italy, *Env. Sci. and Technology*, 2015, 49(9): 5563-70; DU, P. ET AL., Methamphetamine and Ketamine Use in Major Chinese Cities, a Nationwide Reconnaissance through Sewage-Based Epidemiology, *Water Res.*, 2015; KINYUA J. ET AL., Sewage-Based Epidemiology in Monitoring the Use of New Psychoactive Substances: Validation and Application of an Analytical Method Using LC-MS/MS, *see note 8*.

generally expressed in grams or milligrams per day. Often, the obtained value is further divided by the number of inhabitants served by the WWTP, to obtain so called population-normalized or per-capita loads, expressed in milligrams per day per thousand inhabitants. This is generally carried out to account for differences in the size of the population when comparing samples obtained from distinct locations. Another possible step consists in estimating the amount of substance initially consumed by the investigated population.

This procedure, referred to as back-calculation, is carried out using the following equation:

$$\boldsymbol{Consumption}\left[\frac{g}{d}\right] = \left(\boldsymbol{Loads}\left[\frac{g}{d}\right]\frac{1}{\boldsymbol{Excretion}[\%]} * \frac{\boldsymbol{Mwc}}{\boldsymbol{Mwm}}\right) \quad \textbf{Eq. 1}$$

where *Consumption* represents the estimated amount of substance consumed per day, *Loads* are the absolute loads, computed as described above, *Excretion* is the proportion of the initial dose which is found in urine or faeces (or both) as target analyte (e.g., metabolite or parent compound) 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 to back-calculate consumption. Values for *Excretion* are generally derived from clinical trials, where known dosages were administered to volunteers/patients and the composition of their urine (and sometimes faeces) was monitored over time.

3. Implementation

Since its first applications in 2005, WBE has been implemented in numerous locations across the world. Initial studies were based on a limited number of samples collected over short periods. Yet, researchers recognized the need to design and conduct longer and more appropriate campaigns, which would allow a more thorough assessment of changes in drug use. Ever since, the number of studies has increased significantly and routine national and international monitoring campaigns have been set up. Nonetheless, researchers have also recognized the need to combine WBE data to epidemiological, societal, law enforcement, toxicological and/or forensic information, in an attempt to better catch consumption trends, detect the consumption of new substances, and understand how and why such changes are taking place. Such studies have been implemented in

single locations, to better understand the phenomenon in a specific area, but also in multiple locations, in order to include a geographical perspective. Whether in terms of relative (i.e., monitoring drug loads over longer periods and/or comparisons between different locations) or absolute (i.e., back-calculation) quantities, WBE provides useful information about consumption. In fact, it is the sole indicator that really tackles consumption as an entity. Other side of the coin, WBE provides very limited information about consumers (e.g., background of drug use, social and health status), or about the structure of consumption (e.g., injection, frequency of use and polydrug use). Thus, triangulating WBE data with existing epidemiological and/or societal indicators is highly relevant as it allows strengthening the findings from WBE but also gaining additional information about the structure and prevalence of drug use. Data triangulation can definitely trace the path to unveiling original knowledge on illicit consumptions and consumers.

Examples of how data from WBE studies can be combined to other indicators and the types of information that can be gathered have been reported in the literature. In Norway for example, researchers used wastewater data in combination to other data sources to estimate the consumption of cocaine at the population level[24]. Another example was given for amphetamine and methamphetamine. Here, the authors used wastewater and other data sources to determine that methamphetamine consumption increased from 2000 to 2009, but that since then its use had stabilized[25]. In another instance, wastewater data, together with other indicators, was used to investigate differences in drug use among marginalized users in different cities of Norway[26]. Combined with prescription, survey and syringe distribution data, WBE has also been used to evaluate the extent of heroin use in the city of Lausanne[27]. An additional study investigated the consumption of illicit drugs in various cities of Germany and Switzerland

24 REID M. J. ET AL., Estimation of Cocaine Consumption in the Community: A Critical Comparison of the Results from Three Complimentary Techniques, *BMJ Open*, 2012, 2(6).

25 BRAMNESS J. G. ET AL., Recent Trends in the Availability and Use of Amphetamine and Methamphetamine in Norway, *Forensic Sci. Int.*, 2015.

26 AMUNDSEN, E. J. AND REID, M. J., Self-Reports of Consumption of Amphetamines, Cocaine and Heroin in a Survey among Marginalized Drug Users, *Sci. Total Environ.*, 2014.

27 BÉEN F. ET AL., Data Triangulation in the Context of Opioids Monitoring via Wastewater Analyses, *see note 19*.

using a combination of wastewater, survey and crime statistics.[28] Finally, wastewater, police and epidemiological data have been combined using formal statistical models, allowing to estimate various parameters relevant to understanding the extent of drug use (e.g., quantities consumed, prevalence and number of users) and, at the same time, account for the various sources of uncertainty[29]. In Belgium, researchers collected wastewater samples in a specific area and, simultaneously, conducted an online survey to compare the information about illicit drug use derived from the two indicators[30]. The authors found that the outcomes of WBE were in agreement with survey results. Cannabis was confirmed as the most prevalent drug, followed by cocaine, amphetamine and MDMA. Finally, a study carried out in Switzerland recently tackled the added value of wastewater analysis from the perspective of law enforcement[31]. The authors illustrated that combined with information derived from police activity, quantitative data derived from wastewater analysis provided insights into the structure and scale of drug markets at the regional scale.

Nonetheless, the profit of triangulation rests upon the quality of the data which is being used. It is thus essential to thoroughly plan the design of the wastewater sampling campaign, which should be adapted to provide information about national and regional features (potentially by setting up specific local studies). Furthermore, it should allow the integration of other sources of data, ideally having the same level of detail contemplated in the WBE campaign.

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28 BÉEN F. ET AL., Assessing Geographical Differences in Illicit Drug Consumption – A Comparison of Results from Epidemiological and Wastewater Data in Germany and Switzerland, *Drug Alcohol Depend*, 2016.

29 BÉEN F. ET AL., Integrating Environmental and Self-Report Data to Refine Cannabis Prevalence Estimates in a Major Urban Area of Switzerland, *Int. J. Drug Policy*, 2016.

30 VAN WEL, J. H. ET AL., A Comparison between Wastewater-Based Drug Data and an Illicit Drug Use Survey in a Selected Community, *Int. J. Drug Policy,2016*.

31 BÉEN, F., ESSEIVA, P. AND DELÉMONT, O., Analysis of Illicit Drugs in wastewater - Is There an Added Value for Law Enforcement?, *Forensic Sci. Int.*, 2016.