DELIVERABLES 3.1-3.6

D3.1 Report on identification of new synthetic drugs in the illicit drug market

D3.2 Report on measurement of the weekly variation of prescription drugs in sewage

D3.3 Report on identification of metabolic pathways of drugs of abuse in sewage

D3.4 Report on refined estimation methods of population habits

D3.5 Report on reliable estimation of population size and dynamics

D3.6 Report on comparison of results from sewage and social studies in a small and stable community

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PROJECT COORDINATOR: Dr Barbara Kasprzyk-Hordern, University of Bath

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WP3 - Estimation of community-wide health and lifestyle

WP3 aimed to standardize the _sewage epidemiology approach_ and develop novel applications to assess community-wide health and lifestyle. The main objectives were:

- To identify new synthetic drugs of abuse in illicit drug market;

- To identify the abuse of prescription drugs by measuring their weekly variation in wastewater;

- To study metabolic pathways of selected illicit drugs (parent drugs and metabolites) that can occur in sewage/wastewater by microbial metabolism;

- To refine and standardize the sewage epidemiology approach to develop novel applications and compare results with those collected via socio-epidemiological studies

This report summarizes the deliverables related to WP3 highlighting the main research results obtained as publications in peer reviewed journals, and manuscripts in preparation.

The objectives of this WP were fulfilled within the single projects conducted by ESRs/ERs.

Deliverable	Direct contributors	ESR/ER PROJECT TITLE[1]
_D3.1_	**ER4**	_Monitoring the appearance of new synthetic drugs of abuse_
_D3.2_	**ESR10**	_Can variations in drug presence in sewage indicate prescription drug abuse_
_D3.3_	**ESR11**	_Experimental studies for comparison of human metabolism of (new) drugs with microbial metabolism and processes in sewage using sewage samples from different European locations_
_D3.4_ _D3.5_	**ER3**	_Development and standardization of novel sewage epidemiology_ _approaches_
_D3.6_	**ER2**	Epidemiology of drugs – sewage vs. social studies

D 3.1 Report on identification of new synthetic drugs in the illicit drug market

The UK illicit drug market was monitored for the appearance of new compounds promoted for use as recreational drugs. European legislators need to be alerted to the appearance of new compounds that may present health hazards (41 were notified to EMCDDA in 2010). The contents of club amnesty bins provided by UK police forces was monitored on a regular basis. Drugs seized at outdoor music festivals were also analysed. Regular test purchasing of "legal highs" from websites and "head shops" was undertaken. New compounds were characterised by FTIR, GC-MS and where necessary LC-HRMS and NMR. Samples of pooled urine from UK city centre temporary urinals and from music festivals were analysed for the presence of illicit drugs as a sensitive "upstream" indicator to validate and inform other participants working on sewage.

Results

The following results were obtained:

(1) Amnesty bins provided by several police forces as Metropolitan, Avon and Somerset, South Wales, were investigated;

(2) A list of webshops was screened regularly for new products and new compounds were bought;

(3) Pooled urine samples from several festivals and one city centre were collected during the project;

(4) Analytical protocols for drugs were established as follows:

	Powders	Liquids	Herbal	Branded NPS	Tablets and capsules	Other
Step 1	FTIR		Olfactory	TICTAC databases		NA
Step 2	GC-MS					

(5) Analytical protocols for urine analysis were developed: urine was either analysed by previously published methods by a commercial lab (LGC Forensics) and/or analysed by other SEWROF partners;

(6) 46 compounds not previously reported in the UK were detected:

-25 synthetic canabinoids (e.g. F-2201, ADB-FUBINACA, 5F-AMB, etc)

-10 stimulants (e.g. 3-fluorophenmetrazine, bromo-amphetamine, 6-MAPB, etc)

-2 hallucinogens (bk-2CB and N-Methyl-2AI)

-6 benzodiazepines (e.g. flubromazolam, nifoxipam, diclazepam, etc)

-3 depressants (diphenidine, modafiendz, methoxyphenidine)

Other results:

During this project, several in depth studies on certain compounds were conducted:

- monitoring the purity of mephedrone over time;

- assessing the variation of the latest compounds in herbal smoking mixtures;

- development of a single injection quantification method for cocaine;

- metabolisation of 3-fluoro-phenmetrazine (at USAAR).

<u>Publication 1.</u> In this study, 119 mephedrone (4-methylmethcathinone) samples from South Wales were collected between November 2011 and March 2013. Mephedrone purity was determined by gas chromatography–mass spectrometry and cutting agents were identified using Fourier transform infrared spectroscopy (FTIR). Mean mephedrone purity was 68.2 %, with a standard deviation of 24.9 %. A clear time trend was observed, with mephedrone purity declining from 80 % in the first 10 samples collected to 50 % in the last 10 samples collected. The most common cutting agents were monosodium glutamate, creatine, and sucrose.

<u>Publication 2.</u> A suspicious white powder labeled ''idanyl-biphenyl-amninone,'' seized by customs officials at the ''channel island'' of Jersey, UK, was identified and characterized. The elucidation process required the use of several complementary analytical techniques, including gas chromatography–mass spectrometry, liquid chromatography coupled with high-resolution mass spectrometry, nuclear magnetic resonance spectroscopy, and X-ray crystallography. The unknown compound was ultimately identified as 1-(2,3-dihydro-1H-inden-5-yl)-2-phenyl-2-(pyrrolidin-1-yl)-ethanone, a novel cathinone derivative. The results of this study may serve forensic and clinical laboratories in the identification of its related compounds with similar backbone structure using the information reported here.

<u>Publication 3.</u> See publication 3 in D 3.3.

Publications

1. B. Miserez, O. Ayrton, J. Ramsey, Analysis of purity and cutting agents in street mephedrone samples from South Wales, Forensic Toxicology, 32 (2014) 305-310

   http://link.springer.com/article/10.1007/s11419-014-0232-y#page-1

2. Bijlsma, L., B. Miserez, M. Ibanez, J. Ramsey, F. Hernandez, Identification and characterization of a novel cathinone derivative 1-(2,3-dihydro-1 H-inden-5-yl)-2-phenyl-2-(pyrrolidin-1-yl)-ethanone seized by customs in Jersey. , Forensic Toxicology, http://link.springer.com/article/10.1007%2Fs11419-015-0299-0

3. Mardal M, Kinuya J, Ramin P, Miserez B, Maho W, Plósz B, Arndal L, Covaci A, Meyer MR. Screening for DOA in Pooled Human Urine and Urinated Soil Samples and Studies on the Stability of Urinary Excretion Products of Cocaine, MDMA, and MDEA in Waste Water by HILIC LC-MS/MS. http://onlinelibrary.wiley.com/doi/10.1002/dta.1957/abstract

4. A. Frinculescu, C.L. Lyall, J. Ramsey, B. Miserez, Quantification of the synthetic cannabinoids 5F-AKB48 and 5F-PB-22 in smoking mixtures: variation and risks, Drug Test. Anal., accepted for publication

5. B. Miserez, A.L.N. van Nuijs, Waste water analysis for the measurement of population level drug use, SAGE handbook of Drug Research, submitted

6. M. Ibanez, B. Miserez, L. Bijlsma, F. Hernandez, J. Ramsey, Mass spectrometric analysis of new cannabinoids on the UK market since the 2013 legislative ban, Anal. Chim. Acta, submitted

7. Juliet Kinyua; Noelia Negreira; Bram Miserez; Ana Causanilles; Erik Emke; Lies Gremeaux; Pim de Voogt; John Ramsey; Adrian Covaci; Alexander van Nuijs, Qualitative screening of pooled urine samples in Belgium and United Kingdom to map the use of new psychoactive substances, STOTEN, Submitted

8. Mardal, M., Miserez, B., Bade, R., Maurer, H.H., Ramsey, J., Maurer, H.H., Meyer, M.R. 3-fluorophenmetrazine: Studies on its in vivo metabolism in the rat, human and waste water and in vitro metabolism by CYP initial screening, using GC-(HR)-MS and LC-HR-MS/MS techniques, in preparation

9. L. Brockhals, C. Mortsen, J. Ramsey, B. Miserez, Fast and reliable cocaine purity analysis by multiple isotopically labelled internal standard, in preparation

D 2.2 Report on measurement of the weekly variation of prescription drugs in sewage

The primary goal of this project was to look at the weekly variation of concentration of drugs detected in wastewater. This was implemented by developing a statistical model to study the weekly patterns of drugs of abuse vs. not abused drugs. Wastewater-based epidemiology (WBE) has been increasingly used as a novel approach for estimating the amount of use of illicit drugs in a specific geographic area, providing an increasing amount of data each year. However WBE data have mostly been analyzed using simple standard statistical methods. Our aim was to extract more information from WBE data by applying an advanced statistical approach, more specifically functional data analysis (FDA). Once the suitability of FDA was demonstrated this technique was used to review the potential abuse of different medicinal drug groups: benzodiazepines/z-hypnotics and methadone/buprenorphine.

Results

Publication 1. Wastewater-based epidemiology (WBE) is a new methodology for estimating the drug load in a population. Simple summary statistics and specification tests have typically been used to analyze WBE data, comparing differences between weekday and weekend loads. Such standard statistical methods may, however, overlook important nuanced information in the data. In this study, we applied functional data analysis (FDA) to WBE data and compare the results to those obtained from more traditional summary measures. We analyzed temporal WBE data from 42 European cities, using sewage samples collected daily for one week in March 2013. FDA of WBE data extracts more detailed information about drug load patterns during the week which are not identified by more traditional statistical methods.

Publication 2. Application of functional principal component analysis (FPCA) for different illicit drug at the same time in different cities. Functional data analysis (FDA) was applied to study weekly temporal patterns in wastewater curves for six different drugs in Italy. Wastewater samples were collected over seven consecutive days in November 2013, from the inlet of 17 wastewater treatment plants in 17 Italian cities. FPCA extracted detailed features of the weekly temporal patterns of the use of drugs derived from the wastewater analysis. This may help in understanding and monitoring the profile of drug use in a specific community.

Publication 3. See publication 2 in D 3.6.

Publication 4. See publication 3 in D 3.6.

Publication 5. Analysis of caffeine and nicotine as population biomarkers using data from a nation-wide study in Italy. Statistical methods were used to assess also the pattern of use of these substances in Italy. In preparation.

Publication 6. Comparing three advanced statistical methods such as principal component analysis (PCA), FPCA and wavelet-PCA (WPCA) to analyze temporal wastewater data.

Publication 7. FPCA as a method to investigate the potential abuse of prescription drugs.

Publications

1. Salvatore S, Bramness JG, Reid MJ, Thomas KV, Harman C, Røislien J (2015) Wastewater-Based Epidemiology of Stimulant Drugs: Functional Data Analysis Compared to Traditional Statistical Methods. PLoS ONE 10(9): e0138669. doi:10.1371/journal.pone.0138669 . http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0138669

2. Salvatore S, Frøslie KF, Røislien J, Zuccato E, Castiglioni S, Bramness JG: A nuanced picture of illicit drug use in 17 Italian cities through functional principal component analysis of temporal wastewater data. Journal of Public Health. http://link.springer.com/article/10.1007/s10389-016-0717-8.

3. van Wel JHP, Kinyua J, van Nuijs A, Salvatore S, Bramness JG, Covaci A, van Hal G: A comparison between sewage-based epidemiology and epidemiological research in a selected community. International Journal of Drug Policy. http://dx.doi.org/10.1016/j.drugpo.2016.04.003

4. van Wel JHP, Gracia-Lor E, Kinyua J, van Nuijs ALN, Salvatore S, Castiglioni S, Bramness JG, Covaci A, van Hal G: A comparison between wastewater-based epidemiology and epidemiological survey research on alcohol and tobacco use in a selected community. Drug and Alcohol Dependence. doi:10.1016/j.drugalcdep.2016.03.002

5. Gracia-Lor E, Salvatore S, Bramness JG, Zuccato E, Castiglioni S: Comparing consumption of caffeine and nicotine in Italy by wastewater analysis: spatial and temporal patterns of use (in preparation).

6. Salvatore S, Bramness JG, Røislien J: Exploring functional data analysis and wavelet principal component analysis on ecstasy (MDMA) wastewater data. BMC Medical Research Methodology (under review).

7. Salvatore S, Røislien J, Baz Lomba AJ, Bramness JG: Assessing medicinal drug abuse using functional principal component analysis (FPCA) of wastewater data. Pharmacoepidemiology and Drug Safety (submitted).

D3.3 Report on identification of metabolic pathways of drugs of abuse in sewage

To identify human metabolic pathways of new drugs of abuse in sewage, human liver models such as pooled liver microsomes or heterologically expressed human enzymes (CYPs, UGTs, SULTs etc.) were used. In addition, known in vivo data from humans and rats metabolism were considered. If metabolic patterns in sewage from various sources were found to be different, the responsible microbes were identified in collaboration with the institute of microbiology and the metabolic patterns were elucidated by incubating these particular microbes or combinations of them. After drug incubation with human liver models, samples were analyzed using different GC-MS and LC-MS techniques including QqQ, LIT, Orbitrap-Q and LIT-Orbitrap.

Results

(1) Drug incubation with different human enzyme systems or sewage samples;

(2) Incubation of human metabolites with different sewage sample;

(3) Identification of transformation products in these samples and involved microbes;

(4) Comparison of the metabolic patterns.

Publication 1. Biotransformation pathways of the novel psychoactive substance 3,4-methylenedioxypyrovalerone (MPDV) were studied in wastewater (WW) by incubating it, based on the OECD guideline 314 A. Urine and feces collected from rats administered with MDPV were incubated correspondingly. 12 biotransformation products were identified after 10 days of incubation in WW. Three of these biotransformation products were previously reported to be also rat and human metabolites. The presented study indicates that demethylenyl-methyl MDPV, the most abundant metabolite in human urine, should be the best indicator in WW to estimate its use.

Publication 2. The human *in vitro* metabolites of the new synthetic cannabinoid receptor agonist WIN 55,212-2 ((R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone) were studied by using pooled human liver microsomes. Liquid chromatography-high resolution-tandem mass spectrometry (LC-HR-MS/MS) was used for analyses. In total, 19 metabolites were identified indicating a fast and nearly complete metabolism *in vivo*. This is in line with previous findings on other synthetic cannabinoids.

Publication 3. Pooled urine and urinated soil samples collected at festivals were screened for illicit drug excretion products using hyphenated mass spectrometry techniques. The stability of urinary excretion products of cocaine, MDMA, and MDEA in wastewater was also studied. Cocaine and ecstasy-like compounds were the most frequently detected illicit drugs and some metabolites were also identified. A stability test in wastewater was also performed to identify the metabolites most suitable to be used as biomarkers for back-calculation of drug use.

Publications

1. Mardal M, Meyer MR. Studies on the Microbial Biotransformation of the Novel Psychoactive Substance Methylenedioxypyrovalerone (MDPV) in Wastewater by Means of Liquid Chromatography-High Resolution Mass Spectrometry/Mass Spectrometry. Sci Tot Env 493 (2014); 588-595 http://www.sciencedirect.com/science/article/pii/S0048969714008596

2. Mardal M, Garcia Lor E, Leibnitz S, Castiglioni S, Meyer MR. Toxicokinetics of Novel Psychoactive Substances: Plasma protein binding, metabolic stability, and human phase I metabolism of the synthetic cannabinoid WIN 55,212-2 studied by in vitro tools and LC-HR-MS/MS. Drug Testing and Analysis DOI: 10.1002/dta.1938. http://onlinelibrary.wiley.com/doi/10.1002/dta.1938/abstract?userIsAuthenticated=false&deniedAccessCustomisedMessage=

3. Mardal M, Kinuya J, Ramin P, Miserez B, Maho W, Plósz B, Arndal L, Covaci A, Meyer MR. Screening for DOA in Pooled Human Urine and Urinated Soil Samples and Studies on the Stability of Urinary Excretion Products of Cocaine, MDMA, and MDEA in Waste Water by HILIC LC-MS/MS. http://onlinelibrary.wiley.com/doi/10.1002/dta.1957/abstract

4. Mardal M, Bischoff M, Maurer HH, Meyer MR. Microbial Biotransformation of New Psychoactive Substances Using a New Strain Isolated from Waste Water and Raw Waste Water, Structure Identification by LC-QTOF and GC-MS. (*in preparation*)

5. Mardal M, Miserez B, Bade R, Maurer HH, Ramsey J, Meyer MR. 3-fluorophenmetrazine: Studies on its in vivo metabolism in the rat, human and waste water and in vitro metabolism by CYP initial screening, using GC-(HR)-MS and LC-HR-MS/MS techniques (work in progress)

D 3.4 Report on refined estimation methods of population habits

The critical steps of *sewage epidemiology* were evaluated to refine the entire approach and produce homogeneous and reliable data to develop novel applications. These activities will be reported as D3.4 and D3.5.

The first critical step addressed was the current methodology used to estimate drug use which is based on the use of specific correction factors chosen according to pharmacokinetic data of the different substances. A refinement of these correction factors was performed by revising the available information on human metabolism, chiral compounds, route of administration, and doses of consumption.

Results

1. Updated information on human metabolism and preferential route of administration of amphetamine, methamphetamine, MDMA (ecstasy) and cannabis (Tetrahydrocannabinol);
2. Statistical evaluation of excretion data to provide updated mean excretion percentages and refine correction factors
3. Identification of biomarkers of excretion for new psychoactive substances

Publication 1 and 4. The amounts of urinary biomarkers of illicit drugs measured in wastewater are used to back-calculate the consumption of a particular drug by the population. The reliability of back-calculation depends on different factors, one being the accuracy of correction factors. The goal of this study was to refine current correction factors for back-calculation of the most widely used illicit drugs: amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA) and tetrahydrocannabinol (THC). Correction factors were refined for each substance considering the mean percentages of excretion and the most frequent route of administration and were proposed to be used in wastewater based epidemiology to standardize the back-calculation of these drugs.

Publication **Error! Reference source not found.**. See publication 2 in D 3.3.

Publication 3. Analysis of drug residues in urban wastewater could complement epidemiological studies in detecting the use of New Psychoactive Substances (NPS), a continuously changing group of drugs hard to monitor by classical methods. We searched for a selection of 52 NPS potentially used in Italy by using a linear ion trap-Orbitrap high resolution mass spectrometer and few suspects were identified in Italian wastewater samples. We applied both a suspect screening and a target method approach and compared them. The stability of synthetic cannabinoids was also studied in analytical standards and wastewater, identifying the best analytical conditions for future studies.

Publications

1. Sara Castiglioni, Emma Gracia-Lor. Chapter 2. Target drugs residues in wastewater., in: Assessing Illicit Drugs in Wastewater (2016). European Monitoring Centre for Drugs and Drug Addiction, EMCDDA. http://www.emcdda.europa.eu/publications/insights/assessing-drugs-in-wastewater

2. *Mardal M, Garcia Lor E, Leibnitz S, Castriglioni S, Meyer MR. Toxicokinetics of Novel Psychoactive Substances: Plasma protein binding, metabolic stability, and human phase I metabolism of the synthetic cannabinoid WIN 55,212-2 studied by in vitro tools and LC-HR-MS/MS. Drug Testing and Analysis DOI: 10.1002/dta.1938. http://onlinelibrary.wiley.com/doi/10.1002/dta.1938/abstract?userIsAuthenticated=false&deniedAccessCustomisedMessage=*

3. Iria Gonzalez-Marino, Emma Gracia-Lor, Renzo Bagnati, Claudia P.B. Martins, Ettore Zuccato, Sara Castiglioni. Screening New Psychoactive Substances in urban wastewater

using high resolution mass spectrometry. Analytical & Bionanalytical Chemistry, accepted (2016). In press.

4. Emma Gracia-Lor, Ettore Zuccato, Sara Castiglioni. Refining correction factors for back-calculation of illicit drug use. Science of the Total Environment, submitted.

D3.5 Report on on reliable estimation of population size and dynamics

The second critical step of *sewage epidemiology* addressed was related to the estimation of population size and dynamics which is quite important for big cities where daily commuters or other factors may change the number of persons served by a wastewater treatment plant.

The most appropriate estimate of the real population served by a plant was standardized using different approaches such as census data, design capacity of the plant, population equivalent calculated using biological parameters, and amounts of other substances with known consumption (i.e. prescription pharmaceuticals). The use of novel potential substances largely used in a population such as caffeine and nicotine was also explored. Population dynamics (resident or commuter population) was evaluated using data from official statistics.

Results

(1) Caffeine and its main metabolites were tested as new population biomarkers;

(2) Nicotine and its main metabolites were tested as new population biomarkers;

(3) Novel methods to estimate population size and dynamics in a community were established and tested in real scale studies.

Publication 1. The use of caffeine, nicotine and some major metabolites was investigated by wastewater analysis in 13 sewage treatment plants (STPs) across Italy, and their suitability was tested as qualitative and quantitative biomarkers for assessing population size and dynamics. Nicotine metabolites were tested as quantitative biomarkers to estimate population size and the results agreed well with census data. Caffeine and its metabolites were confirmed as good qualitative biomarkers, but additional information is needed on the caffeine metabolism in relation to the multiple sources of its main metabolites.

Publication 2. See WP2 report. ESR 9.

Publication 3. See Publication 3 in D 3.6.

Publications

1. Ivan Senta, Emma Gracia-Lor, Andrea Borsotti, Ettore Zuccato, Sara Castiglioni. Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment. Water Research 74 (2015) 23-33. http://www.sciencedirect.com/science/article/pii/S0043135415000755

2. Richard Bade, Nikolaos I. Rousis, Lubertus Bijlsma, Emma Gracia-Lor, Sara Castiglioni, Juan V. Sancho, Félix Hernández. *Screening of pharmaceuticals and illicit drugs in wastewater and surface waters of Spain and Italy by High Resolution Mass Spectrometry using UHPLC-QTOF MS and LC-LTQ Orbitrap MS.* Analytical and Bioanalytical Chemistry (2015) 407:8979–8988.

3. JHP van Wel, E Gracia-Lor, ALN van Nuijs, J Kinyua, S Salvatore, S Castiglioni, J Bramness, A Covaci, G Van Hal. Investigation of agreement between wastewater-based epidemiology and survey data on alcohol and nicotine use in a community. *Drug and alcohol analysis (2016).* doi:10.1016/j.drugalcdep.2016.03.002

4. Georgia Gatidou, J. Kinyua, A.L.N. van Nuijs, E. Gracia-Lor, S. Castiglioni, A. Covaci, A.S. Stasinakis. Drugs of abuse and alcohol consumption among different groups of population on the Greek island of Lesvos through sewage-based epidemiology. Science of the Total Environment, accepted for publication.

5. Jose Antonio Baz-Lomba, Stefania Salvatore, Emma Gracia-Lor, Yeonsuk Ryu, Malcolm Reid, Sara Castiglioni, Jørgen G. Bramness, Kevin V. Thomas. Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. Environmental Research, submitted

6. Emma Gracia-Lor, E. Zuccato, S. Castiglioni. Caffeine intake estimation through the analysis of caffeine metabolites in wastewater. In preparation

7. Yeonsku Ryu, Emma Gracia-Lor, Malcolm Reid, Sara Castiglioni, Jørgen G. Bramness, Kevin. V. Thomas. Analysis of oxidative stress biomarker 8-iso-prostaglandin F2α in wastewater and its correlation with legalised drugs. In preparation

D3.6 Report on comparison of results from sewage and social studies in a small and stable community

A study to correlate *sewage epidemiology* results with socio-epidemiological findings was carried out. This comparison was performed investigating drug use via analysis of sewage in a relatively small and stable community, e.g., with limited 'movement flow', such as a small-medium city (50,000 – 100,000 inh.) to ensure a higher degree of confidence in the sewage results and in the correlation with the community inhabitants. The population survey was developed containing questions on substance use which are already employed in the Belgian Health Interview Survey, EMCDDA, WHO surveys and other relevant surveys. All inhabitants received a questionnaire with validated questions on recent drug use. Ethical approval was applied for at the Ethics Committee of the University Hospital of Antwerp.

Results

Publication 1. The goal of the current study was to compare the results from wastewater-based epidemiology (WBE) and from more conventional epidemiological techniques. A website was opened during a 12-week period (autumn 2014) on which inhabitants of a selected community (N=29,083) were asked to indicate their drug use in the past week. Concomitant wastewater samples were taken from the wastewater treatment plant (WWTP) collecting from the community. Results from both the survey and wastewater study matched national and international trends, but caution should be exercised in combining the two approaches. Future research on combining the two approaches should

focus on either a more general approach, e.g. national population surveys, or take place in a more focused setting.

Publication 2. The current study evaluate using wastewater-based epidemiology (WBE) for assessing illicit drug use by comparing wastewater data with that from a population survey. According to the survey results cannabis was the most used drug followed by amphetamine, cocaine and MDMA.

Wastewater data corroborated these results. Cocaine, amphetamine and MDMA showed a significant difference between days of the week. Results from both the survey and wastewater study matched national and international trends. Wastewater analysis confirm that WBE can be reliably used to confirm patterns and trends in drug use.

Publication 3. This study compares estimates of nicotine and alcohol use from a wastewater sampling campaign in a medium-sized Belgian city with a concurrently executed population survey. Ethylsulfate was used as alcohol biomarker and cotinine and trans-3'-hydroxycotinine as nicotine biomarker. According to survey data, alcohol and nicotine were used less than in the rest of Belgium and this was matched by the wastewater data. Nicotine use, but not alcohol use, showed a significant variation over the sampling periods. Both nicotine and alcohol showed increase use during the weekend while only alcohol showed a different use pattern throughout the week. No correlation between WBE and survey data could be demonstrated, possibly due to small sample sizes.

Publications

1. van Wel JHP, Kinyua J, van Nuijs A, van Hal G, Covaci A (2015). Methodological considerations for combining wastewater-based epidemiology with survey research. Archives of Public Health, 73(Suppl 1):P29.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4582311/

2. van Wel JHP, Kinyua J, van Nuijs A, Salvatore S, Bramness JG, Covaci A, van Hal G (2016). A comparison between wastewater-based drug data and an illicit drug use survey in a selected community. *International Journal of drug Policy*; In press. 10.1016/j.drugpo.2016.04.003.

3. van Wel JHP, Gracia-Lor E, Kinyua J, van Nuijs ALN, Salvatore S, Castiglioni S, Bramness JG, Covaci A, van Hal G (2016). Investigation of agreement between wastewater-based epidemiology and survey data on alcohol and nicotine use in a community. *Drug and Alcohol Dependence,* 162; 170-175.
http://www.sciencedirect.com/science/article/pii/S037687161600137X

Milan, 26/04/2016

Dr. Sara Castiglioni, leader WP3