



## Cocaine and metabolites in waste and surface water across Belgium

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*Cocaine consumption can be evaluated through analysis of waste and surface water.*

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### ABSTRACT

Cocaine abuse, a growing social problem, is currently estimated from population surveys, consumer interviews and crime statistics. A new approach based on the analysis of cocaine (COC) and metabolites, benzoylecgonine (BE) and ecgonine methyl ester (EME), in water samples was applied to 28 rivers and 37 waste water treatment plants in Belgium using solid-phase extraction and liquid chromatography coupled to tandem mass spectrometry. While EME was undetectable, COC and BE were detectable with concentrations ranging from <1 to 753 ng/L and <1 to 2258 ng/L, respectively. BE concentrations were employed to calculate the local amount of abused cocaine. The highest values (up to 1.8 g/day cocaine per 1000 inhabitants) were found in large cities and during weekends. The estimation of cocaine abuse through water analysis can be executed on regular basis without cooperation of patients. It also gives clear geographical information, while prevention campaigns can easily be implemented and evaluated.

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### 1. Introduction

Abuse of illicit drugs is a major problem in our contemporary society. The consumption of drugs leads not only to a high mortality and morbidity, but also to many socio-economic problems (economic damage, criminality, social isolation, a.s.o.). The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) estimates that even 4% of the European adults (approximately 12 million people) has used cocaine (COC) during their lifetime, and that 3 million European adults have used cocaine in 2007 (EMCDDA, 2007). The EMCDDA observed, after a stabilizing trend in 2006, an obvious increase in cocaine consumption during 2007. This explains why cocaine abuse seems to be a growing problem in Europe and a major issue for the European drug policy. By comparison, a stabilizing and even decreasing trend for the consumption of other drugs such as ecstasy and cannabis has been observed (EMCDDA, 2007), probably as a consequence of their replacement by cocaine. A decrease in the cocaine price could be another explanation. These trends in drug abuse are estimated indirectly from population surveys, consumer interviews,

individual medical records and crime statistics (EMCDDA, 2002). Such general indicators can give only raw estimations of drug abuse, but accurate and local measurements are not possible with this approach (Zuccato et al., 2005). Also in Belgium, limited information is available about the cocaine abuse (Lamkaddem and Roelands, 2007).

Cocaine is still administered as topical anesthetic compound in intranasal, ophthalmologic and bronchoscopic procedures (Dart, 2004). Illegally, cocaine is used by intranasal ("snorting"), intravenous or smoking routes (Cone, 1995). It has three important actions in humans: direct blocking of fast sodium channels, interfering with the uptake of neurotransmitters such as epinephrine and dopamine and a vasoconstrictive action (Dart, 2004). The use of cocaine has direct physiological effects on humans, such as central nervous system over-stimulation, risk of heart attack, pulmonary complications and altered serotonin levels (Lakoski et al., 1991). In humans, COC is rapidly hydrolyzed to benzoylecgonine (BE) and ecgonine methyl ester (EME) and some minor metabolites (Dart, 2004). Only a small fraction of COC is excreted in urine as parent compound, while the largest amount is excreted as BE, the most important metabolite, and EME (Ambre et al., 1984, 1988). In urine, COC can be detected for approximately 8 h, while BE and EME can be positively identified for more than 96 h (Hamilton et al., 1977; Jufer et al., 2000).

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Recently, a new, direct and objective way of monitoring drug consumption has been proposed, based on the measurement of urinary excreted drugs and their metabolites in waste and surface water (Bones et al., 2007; Castiglioni et al., 2006; Gheorghe et al., 2008; Huerta-Fontela et al., 2007; Hummel et al., 2006; Kasprzyk-Hordern et al., 2008a,b; Zuccato et al., 2005, 2008). While these studies are particularly based on measurements by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS), they differ on the sample preparation approaches and the groups of investigated drugs of abuse.

In the present study, a previously optimized and validated method based on solid-phase extraction (SPE) and hydrophilic interaction (HILIC) LC–MS/MS (Gheorghe et al., 2008) was applied to quantify COC, BE and EME in waste water from 37 waste water treatment plants (WWTPs) and in surface water from 28 rivers and brooks across Belgium. To the best of the authors' knowledge, this is the largest monitoring study of cocaine and its metabolites in waste and surface waters up to date. The concentrations of COC and metabolites were then converted into cocaine equivalents, using the flow rate of the water stream and a formula that takes into account the molecular masses and the excretion pattern of cocaine and metabolites. The final aim was to detect regions with a high consumption of cocaine. This approach will ultimately lead to valuable information needed to tackle the growing problem of cocaine abuse in Belgium and in Europe.

## 2. Materials and methods

### 2.1. Sampling sites and sample collection

#### 2.1.1. Sampling sites

WWTPs ( $n = 37$ ) were chosen based on the amount of persons they serve and on their geographical location, so that the overall picture of cocaine use in Belgium could be made (Table 1, Fig. 1 and Fig. SI-1). Small WWTPs, which serve less than 10 000 persons, were excluded from this study. The chosen WWTPs cover around 3 700 000 inhabitants (~40% of the total Belgian population). Sampling sites on 28 rivers and brooks ( $n = 43$ ) were also chosen so that they cover the whole Belgian hydrographic system (Table 2 and Fig. SI-2). Two rivers (the Demer and the Dijle) were sampled during the same day at four different downstream sites to evaluate differences in concentrations of COC and BE. One river, the Zenne, was sampled upstream and downstream of the urban region of Brussels.

#### 2.1.2. Sample collection

Influent water samples (24-h flow dependent composite samples, 1.5 L) were collected from WWTPs across Belgium. For each WWTP, two samples were taken, one on Sunday and one on Wednesday to evaluate differences in cocaine abuse during the week. From the WWTP of Mouscron (4 Nov 07) we received a 99-h composite sample, from Tournai (20 Jan 08) a 48-h composite sample and from Waver (18 Nov 07) a 72-h composite sample. Surface water samples (grab samples, 2.5 L) were collected at 43 sampling points from 28 rivers and brooks across Belgium. All samples were collected during periods with low or no rainfall to prevent excessive dilution of analytes. Only for river Ijzer, the sampling was conducted after a heavy rainfall. For each sample, pH and temperature at the time of sampling were recorded.

All samples were collected in glass bottles, adjusted to pH = 2 with 37% hydrochloric acid (HCl) and stored at  $-20^{\circ}\text{C}$  until analysis. The pH adjustment was found to be necessary to prevent degradation of COC, BE and EME during transport and storage (Gheorghe et al., 2008). The sample collection took place from June 2007 until February 2008.

### 2.2. Reagents and materials

The compounds of interest (COC, BE and EME) and their deuterated analogues (COC- $d_3$ , BE- $d_3$  and EME- $d_3$ ), used as internal standards, were of analytical grade (purity > 98%) and were purchased from Cerilliant (Round Rock, TX, USA) at concentrations of 100 ng/ $\mu\text{L}$  in methanol (MeOH). Working mixtures of the standards with concentrations ranging between 0.01 and 10 ng/ $\mu\text{L}$  were prepared in MeOH. LC-grade acetonitrile (AcN) and MeOH were purchased from Merck (Darmstadt, Germany). Milli-Q water was obtained by purifying demineralised water in a Milli-Q system (Millipore, Bedford, MA, USA). Chloric acid (HCl) and ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) were also obtained from Merck. Oasis HLB<sup>®</sup> SPE cartridges (6 mL, 500 mg) were purchased from Waters (New Bedford, MA, USA). A Supelco Visiprep<sup>™</sup> SPE Vacuum Manifold (Supelco, Bellefonte, Pennsylvania, USA) with 12 ports and a self-cleaning dry vacuum were used for the loading of water samples onto the SPE cartridges, the drying of the cartridges and the elution of analytes.

### 2.3. Analytical method

Cocaine and metabolites were analyzed in waste and surface water samples as previously described (Gheorghe et al., 2008). Before extraction, water samples (100 mL for WWTPs, 500 mL for rivers and brooks) were filtered over a glass microfiber filter ( $\varnothing$  240 mm, Macherey-Nagel, Düren, Germany) to remove solid particles. Afterwards, samples were adjusted to pH = 6 with  $\text{NH}_4\text{OH}$  and then the corresponding deuterated internal standards (100 ng BE- $d_3$  and 20 ng COC- $d_3$  for waste water or 50 ng BE- $d_3$  and 10 ng COC- $d_3$  for surface water) were added. Oasis HLB<sup>®</sup> cartridges, consisting of a divinylbenzene/*N*-vinylpyrrolidone copolymer with both hydrophilic and lipophilic properties, were conditioned with 3 mL of MeOH and 3 mL of Milli-Q water. Samples were loaded on the cartridges at a flow rate of 3 mL/min. The cartridges were then washed with 3 mL of a 5% MeOH in Milli-Q water solution, vacuum-dried for 15 min and eluted with  $2 \times 4$  mL of MeOH. The eluates were dried under a gentle nitrogen stream and the dried samples were reconstituted in 150  $\mu\text{L}$  AcN/MeOH mixture (3/1, v/v). The reconstituted samples were filtered through modified nylon membrane (0.45  $\mu\text{m}$  pore size) and centrifuged for 5 min. The filtrate was then transferred to a glass vial for analysis.

Samples were analyzed by an Agilent 1100 series HPLC system equipped with binary pump and autosampler (Palo Alto, CA, USA), using a ZORBAX Rx-SIL HILIC column (2.1 mm  $\times$  150 mm, 5  $\mu\text{m}$ ) (Agilent) and a guard column with the same stationary phase. The LC was coupled to an Agilent 1100 Series MSD ion trap with electrospray ionization (ESI) operated by an HP Chemstation for MS control and spectral processing. The ESI interface was operated in positive ionization mode. MS analyses were performed in multiple reaction monitoring (MRM) mode by measuring the product ions formed by the fragmentation of the protonated pseudomolecular ions of COC, BE and EME and their internal standards.

### 2.4. Quality control – quality assurance

The method used in the present study was validated as previously described (Gheorghe et al., 2008). Calibration curves for all three compounds were generated and the intermediate precision, trueness and limit of quantification (LOQ) were determined. A summary of the method validation is given in Table SI-1. The identity for each of the investigated compounds in surface and waste water samples was confirmed through matching of retention times (relative to the standard and to the deuterated analogues used as internal standards) and on the specific MRM monitored for each analyte (Gheorghe et al., 2008).

An interlaboratory test was performed between the two participating laboratories, the Toxicological Centre (TC) in Antwerp and the Laboratory for Environmental Toxicology (LET) in Liège to evaluate the protocol used for analysis of COC, BE and EME. Nine waste water samples were exchanged and were analyzed in triplicate (for TC) or in single (for LET) following a similar protocol. Minor modifications for the analysis of COC and metabolites in waste and surface water were made by the LET: (i) the use of a  $\text{C}_{18}$  UPLC column and (ii) the use of an UPLC–MS/MS instrument. A tap water sample spiked with 200 ng/L COC and 800 ng/L BE was also analyzed by the two laboratories. Table SI-2 summarizes the results for the interlaboratory test. The relative standard deviation (RSD) of the mean concentrations of COC and BE analyzed by the two laboratories was in all cases lower than the recommended cut-off value of 20% (Reed et al., 2002). This is for the first time that an interlaboratory test is reported for the analysis of COC and metabolites in waste water.

A quality control was executed during the analyses. With each batch of seven samples, a tap water sample spiked with 206 ng/L COC and 841 ng/L BE was analyzed and the recovery was calculated. Results are visualised in Figs. SI-3 and SI-4. All quality controls are within the control limits of  $3 \times \text{SD}$ , as suggested by Masson (2007).

## 3. Results and discussion

### 3.1. Assumptions to calculate cocaine equivalents

To evaluate the cocaine abuse in the selected regions, it is necessary to transform the concentrations (in ng/L) into total cocaine equivalents (in g/day) and, if possible, to further report these cocaine equivalents per 1000 inhabitants. For these calculations, different parameters which play an important role have to be known: (i) the flow rate of the water to transform concentrations of COC and BE into loads of COC and BE (expressed in g/day); (ii) the relative amount of a cocaine dose excreted as COC and BE to make a back calculation from loads COC and BE to a total amount of abused COC (in g/day); (iii) the molecular masses of the different compounds/metabolites; (iv) the stability (temperature, pH and time of presence in water) of COC and metabolites in aqueous matrices; and (v) the amount of inhabitants that is served by a certain WWTP.

In the literature two approaches to calculate cocaine equivalents can be found. Zuccato et al. (2005) proposed a formula based

**Table 1**  
Results for waste water samples

WWTP	Sampling date		Flow rate (m <sup>3</sup> /s)		# Inhabitants	Conc. BE (ng/L)		Conc. COC (ng/L)		Loads BE (g/day)		Loads COC (g/day)		COC eq (g/day per 1000 inh.)		Ratio COC/BE	
	Sun	Wed	Sun	Wed		Sun	Wed	Sun	Wed	Sun	Wed	Sun	Wed	Sun	Wed	Sun	Wed
Aalst	12/08/07	15/08/07	0.220	0.229	89 847	322	455	92	128	6.1	9.0	1.7	2.5	0.158	0.233	0.27	0.27
Aartselaar	26/08/07	29/08/07	0.228	0.217	61 520	365	327	118	66	7.2	6.1	2.3	1.2	0.272	0.232	0.31	0.19
Antwerpen-Noord	19/08/07	29/08/07	0.254	0.244	69 668	515	358	149	127	11.3	7.5	3.3	2.7	0.377	0.252	0.28	0.34
Antwerpen-Zuid	16/12/07	05/12/07	0.769	1.109	157 268	1858	894	584	324	123.5	85.7	38.8	31.1	1.829	1.269	0.30	0.35
Arlon	13/01/08	09/01/08	0.173	0.165	16 043	351	315	117	71	5.3	4.5	1.8	1.0	0.762	0.653	0.32	0.21
Waver	18/11/07	14/11/07	0.210	0.299	80 000	248	149	60	28	4.5	3.9	1.1	0.7	0.131	0.112	0.23	0.18
Beersel	14/10/07	05/12/07	0.155	0.333	63 531	527	171	187	53	7.1	4.9	2.5	1.5	0.259	0.180	0.34	0.30
Brugge	23/12/07	03/10/07	0.833	1.990	199 018	324	53	87	20	23.3	9.1	6.3	3.5	0.298	0.098	0.26	0.37
Brussel-Noord	16/12/07	19/12/07	2.932	2.836	850 000	1291	909	348	273	327.0	222.7	88.0	66.8	0.896	0.610	0.26	0.29
Dendermonde	28/10/07	19/09/07	0.184	0.255	68 276	1221	504	230	110	19.4	11.1	3.7	2.4	0.663	0.379	0.18	0.21
Destelbergen	14/10/07	05/12/07	0.155	0.340	57 999	698	161	138	50	9.4	4.7	1.9	1.5	0.376	0.190	0.19	0.30
Deurne	19/08/07	29/08/07	0.519	0.582	198 569	2258	1610	753	703	101.3	80.9	33.8	35.3	1.189	0.949	0.32	0.42
Tournai	20/01/08	16/01/08	0.214	0.411	29 000	211	46	52	10	3.9	1.6	1.0	0.4	0.313	0.132	0.23	0.21
Genk	16/12/07	17/10/07	0.504	0.492	68 294	538	866	230	287	23.5	36.8	10.0	12.2	0.792	1.243	0.41	0.32
Gent	12/08/07	15/08/07	0.601	0.586	206 109	801	516	232	198	41.6	26.1	12.0	10.0	0.470	0.295	0.28	0.37
Harelbeke	23/12/07	26/09/07	0.408	0.972	111 515	751	304	271	97	26.5	25.5	9.6	8.1	0.553	0.533	0.34	0.30
Hasselt	16/12/07	17/10/07	0.397	0.376	63 333	402	467	134	126	13.8	15.2	4.6	4.1	0.507	0.559	0.32	0.26
Heist	20/01/08	24/10/07	0.133	0.086	21 196	296	716	80	141	3.4	5.3	0.9	1.1	0.314	0.718	0.26	0.19
Leuven	21/10/07	17/10/07	0.240	0.395	113 015	1045	339	220	105	21.6	11.6	4.6	3.6	0.446	0.238	0.20	0.30
Liedekerke	28/10/07	19/09/07	0.192	0.258	92 465	811	392	253	89	13.4	8.7	4.2	2.0	0.338	0.220	0.30	0.22
Liège	13/01/08	09/01/08	0.082	0.118	26 300	569	206	131	69	4.0	2.1	0.9	0.7	0.358	0.187	0.22	0.32
Lokeren	12/08/07	15/08/07	0.198	0.240	37 199	334	343	112	152	5.7	7.1	1.9	3.2	0.357	0.445	0.32	0.42
Mechelen-Noord	21/10/07	05/12/08	0.157	0.450	87 452	1994	379	515	256	27.1	14.7	7.0	9.9	0.721	0.393	0.25	0.64
Menen	04/11/07	26/09/07	0.145	0.673	62 575	594	173	179	47	7.4	10.1	2.2	2.7	0.277	0.375	0.29	0.26
Mol	28/10/07	12/12/08	0.124	0.191	47 538	665	163	190	30	6.8	2.7	1.9	0.5	0.332	0.132	0.27	0.18
Charleroi	18/11/07	14/11/07	0.473	0.533	138 000	994	839	370	242	40.6	38.6	15.1	11.1	0.686	0.652	0.36	0.28
Morkhoven	28/10/07	12/12/07	0.078	0.301	38 211	485	140	88	44	3.3	3.7	0.6	1.1	0.200	0.223	0.17	0.30
Mouscron	15/10/07	03/10/07	0.039	0.200	27 831	143	109	32	32	0.5	1.9	0.1	0.6	0.040	0.158	0.21	0.28
Nivelles	16/12/07	19/12/07	0.167	0.167	27 000	350	392	96	141	5.0	5.6	1.4	2.0	0.435	0.487	0.26	0.34
Oostende	10/02/08	03/10/07	0.487	1.002	128 822	312	181	121	57	10.5	15.9	4.1	5.0	0.189	0.290	0.37	0.30
Roeselare	23/12/07	26/09/07	0.233	0.580	62 438	380	131	128	32	7.7	6.5	2.6	1.6	0.285	0.244	0.32	0.23
La Louvière	13/01/08	14/11/07	0.238	0.205	29 800	328	156	95	38	6.8	0.7	2.0	0.7	0.528	0.217	0.28	0.23
Sint-Niklaas	12/08/07	15/08/07	0.172	0.298	44 443	347	428	115	79	5.2	11.0	1.7	2.0	0.270	0.578	0.32	0.18
Sint-Truiden	16/12/07	24/10/07	0.291	0.192	44 131	189	316	59	88	4.7	5.3	1.5	1.5	0.250	0.277	0.30	0.27
Mons	18/11/07	14/11/07	0.095	0.230	82 350	365	249	115	83	8.5	5.0	2.7	1.7	0.241	0.140	0.30	0.32
Verviers	13/01/08	09/01/08	0.716	0.680	95 000	261	187	75	63	16.2	11.0	4.7	3.7	0.396	0.269	0.28	0.32
Wulpen	04/11/07	03/10/07	0.180	0.237	43 200	547	94	111	23	8.5	1.9	1.7	0.5	0.418	0.092	0.19	0.23

WWTP = waste water treatment plant, BE = benzoylecgonine, COC = cocaine, COC eq = cocaine equivalents, Sun = Sunday, Wed = Wednesday, inh = inhabitants. The COC eq were calculated based on the following formula: COC eq = 2.33 × loads BE, as proposed by Zuccato et al. (2005).

exclusively on BE loads. They assumed that about 45% of a cocaine dose is excreted as BE and only a small fraction as COC. Bones et al. (2007) calculated the cocaine equivalents based exclusively on COC loads and on the assumption that approximately 10% of a cocaine dose is excreted as COC. This group assumed also that COC is stable in waste and surface water and that the source of the COC found in waste and surface water comes from human excretion rather than from dumping of large quantities. The latter approach seems to be less reliable than the one proposed by Zuccato et al. (2005), since COC degraded rapidly in water, while BE is more stable (Gheorghe et al., 2008).

The above-described approaches (Bones et al., 2007; Zuccato et al., 2005) are based on several assumptions and they probably underestimate the real use of cocaine. The literature is inconsistent regarding the urinary excretion of COC. The relative amount of a COC dose excreted as COC and BE differs strongly between various experimental setups. Between 1 and 15% of a cocaine dose is excreted as COC, while between 15 and 55% of a cocaine dose is excreted as BE (Ambre et al., 1984, 1988; Cone et al., 1998). In the future, it is necessary to develop a new mathematical model that takes every process (stability in water, urinary excretion, adsorption on solid particles) into account for a more exact estimation of the amount of cocaine abused in a certain region.

The calculation of the cocaine equivalents in Tables 1 and 2 is performed by applying the formula by Zuccato et al. (2005). In Table 1, the calculation of the cocaine equivalents per 1000 inhabitants is made.

### 3.2. Cocaine and metabolites in surface water across Belgium

Table 2 and Fig. SI-2 summarize the concentrations of COC and BE and their corresponding COC equivalents in surface water across Belgium. In all samples, EME was found below LOQ (20 ng/L), probably because of its low stability in water (Gheorghe et al., 2008) and its high LOQ compared to BE and COC, and therefore was not included in further calculations. COC and BE were present in surface water with a maximum of 115 and 520 ng/L, respectively. For surface water, no further association between the COC equivalents and the amount of inhabitants living on the river's basin was made, since the latter cannot be estimated with accurate precision.

Generally, COC and BE levels were above LOQ in most surface water samples from Flanders (North of Belgium). Only for some smaller rivers, such as the Binchebeek and the Dommel, COC or BE was below LOQ. The absence of COC and BE in the Ijzer, a rather large river, is probably because of the massive rainfall during the sampling period (Table 2), which resulted in a high dilution of COC and BE in the surface water. Contrarily, we had a lower number of positive samples in surface water from Wallonia (South of Belgium), most probable due to the lower population density in Wallonia compared to Flanders.

The Dijle, a river that rises in Wallonia, was sampled at four different sites: Limelette, Florival, Korbeek and Wilsele (from upstream to downstream). An increasing trend in the COC equivalents was observed when going downstream the Dijle. This

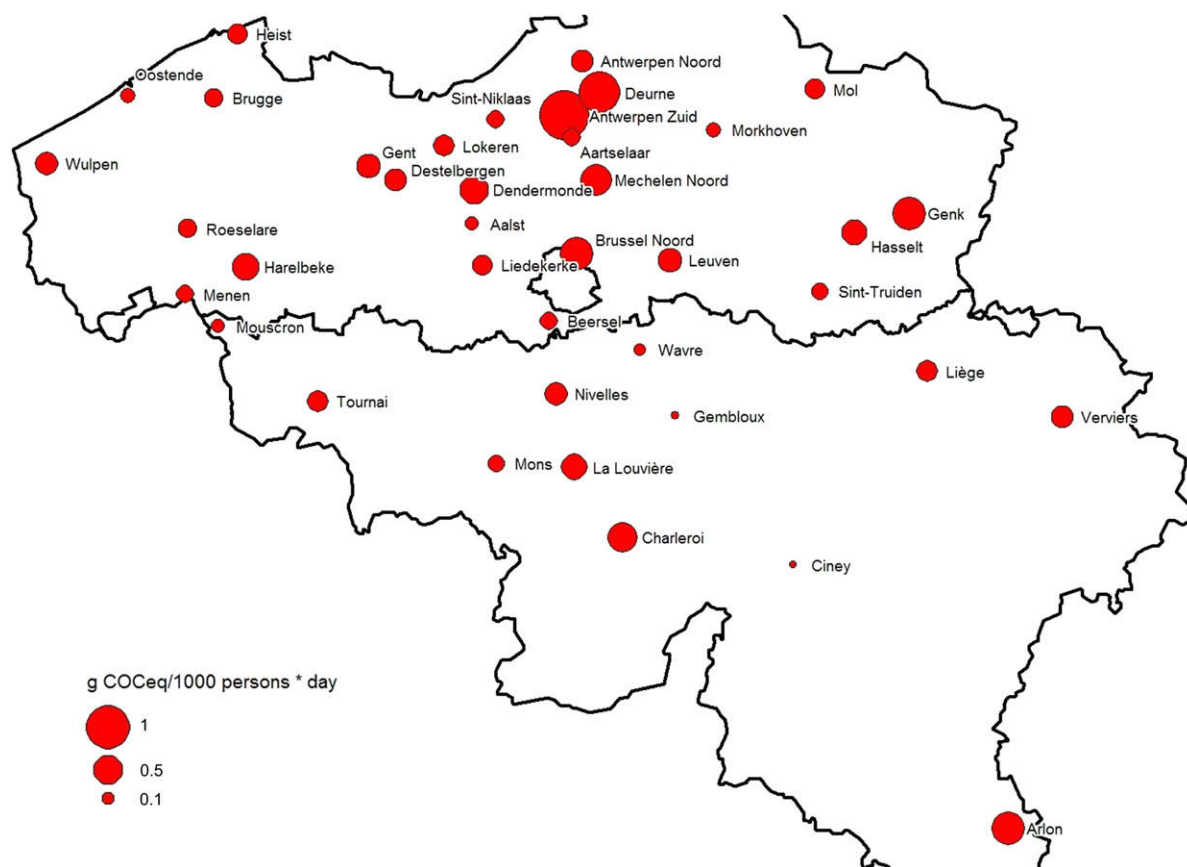


Fig. 1. Locations and results (in COC equivalents in g/day per 1000 inhabitants) of WWTP water samples collected during weekend.

observation agrees with earlier reports in other rivers (Zuccato et al., 2008) and could be explained by the continuous contamination of the rivers by waste water resulting from domestic use. Most of the domestic water runs through WWTPs, with variable removal efficiencies of COC and metabolites largely depending on the applied treatment technique (Castiglioni et al., 2006). Therefore, the effluent of the WWTPs may still contain COC and BE. Moreover, direct discharges of untreated waste water in the rivers are possible, since a large percentage of the population is not connected to a WWTP. The small drop in COC equivalents between Korbeek and Wilsel is probably a consequence of the fact that the Dijle does not receive waste water in this area and that, in the absence of additional source, the degradation of COC and BE occurs in water (Gheorghe et al., 2008). A similar trend in the COC equivalents was seen for the Demer, with the following sampling sites ranked downstream: Hasselt, Kermt, Halen and Aarschot.

Concentrations of COC and BE found in Belgian surface waters are in most cases comparable with concentrations measured in Italy (Zuccato et al., 2005, 2008), Spain (Huerta-Fontela et al., 2007) and the UK (Kasprzyk-Hordern et al., 2008a,b; Zuccato et al., 2005) (Table 3).

Since COC is transformed readily in BE in the human body, a higher concentration of BE than COC in surface water could be expected. This was confirmed by our results and results from the other studies, in which the logical pattern of higher BE concentrations than COC concentrations is observed. However, our results differ from those of Bones et al. (2007), who have detected COC in surface water without being able to measure BE in most cases. Therefore, this is a remarkable (if not questionable) finding which has been partially explained by Bones et al. through the use of a different sample preparation technique.

The mean  $\pm$  SD ratio COC/BE for the surface water samples was  $0.18 \pm 0.11$  and no outliers were observed. This value agrees well

with values reported for urinary excretion of BE and COC (mean 8% COC, 35% BE; COC/BE = 0.23) (Ambre et al., 1984, 1988; Cone et al., 1998). The relatively high RSD (60%) is a consequence of the very broad ranges of urinary excretion of COC and BE reported in the literature. Our findings are comparable with, but yet higher than the ratio measured by Zuccato et al. (2005) in the Po river (0.05), probably attributed to different temperatures of river Po and the rivers sampled in the present study, resulting in a different degradation pattern of COC and BE.

The concentrations of BE and COC in the river Zenne at Vilvoorde (downstream of Brussels) are well above the levels observed in other Belgian rivers (this study) and in other European rivers (Table 3). The river Zenne still receives directly a large amount of industrial and domestic untreated waste water from Brussels, which explains the high concentrations of COC and BE observed downstream of Brussels. This hypothesis is confirmed by the low levels of COC and BE measured in the surface water collected on the same day from river Zenne in Lot (upstream of Brussels) (Table 2), which suggests that indeed the urban agglomeration of Brussels is responsible for the dramatic increase in concentrations of COC and BE in the water of river Zenne.

### 3.3. Cocaine and metabolites in waste water across Belgium

COC and BE were quantifiable in all waste water samples collected from WWTPs spread across Belgium (Table 1 and Fig. SI-1). Similar to surface water, EME was below LOQ (20 ng/L) in all WWTP samples. It is worth mentioning that EME was neither reported nor measured in WWTP samples until now. The concentration ranges of COC and BE in the influent WWTP water samples were 10–753 ng/L and 33–2258 ng/L, respectively.

**Table 2**  
Results for surface water samples

River	Town/village	Sampling date	Flow rate (m <sup>3</sup> /s)	Concentration BE (ng/L)	Concentration COC (ng/L)	Loads BE (g/day)	Loads COC (g/day)	COC eq (g/day)	Ratio COC/BE
Aa	Oud-Turnhout	27/06/07	0.841	8.6	4.1	0.6	0.3	0.46	1.453
Aa	Poederlee	27/06/07	2.500	10.1	1.2	2.2	0.	0.11	5.065
Amblève	Martinrive	12/10/07	8.726	<0.5	<1	–	–	–	–
Bellebeek	Ternat	10/06/07	0.125	75.1	12.2	0.8	0.1	0.15	1.889
Binchebeek	Geraardsbergen	27/07/07	nm	<0.5	<1	–	–	–	–
Demer	Halen	26/07/07	2.100	47.6	12.6	8.6	2.3	0.25	20.098
Demer	Aarschot	26/07/07	9.079	38.5	7.6	30.2	6.0	0.19	70.316
Demer	Hasselt	26/07/07	1.281	44.3	13.2	4.9	1.5	0.28	11.415
Demer	Kermt	26/07/07	3.515	32.2	8.3	9.8	2.5	0.25	22.813
Dender	Overboelare	10/06/07	1.555	23.4	3.0	3.2	0.4	0.12	7.337
Dender	Dendermonde	27/07/07	0.613	8.1	<1	0.4	–	–	1.002
Dendre Occ.	Ligne	27/07/07	0.234	2.3	<1	0.1	–	–	0.106
Dijle	Wilsele	17/07/07	9.698	27.6	2.2	23.1	1.8	0.08	53.881
Dijle	Korbeek	17/07/07	9.98	34.2	5.8	29.5	5.0	0.16	68.702
Dijle	Limelette	26/07/07	2.787	21.5	2.4	5.19	0.6	0.10	12.083
Dijle	Florival	17/07/07	2.940	23.9	3.1	6.1	0.8	0.12	14.143
Dommel	Neerpelt	17/07/07	1.085	<0.5	<1	–	–	–	–
Grote Molenbeek	Sint-Amands	22/06/07	0.219	190.5	26.2	3.6	0.5	0.13	8.397
Grote Nete	Hulshout	27/06/07	5.414	8.2	1.2	3.9	0.6	0.14	8.988
Grote Nete	Geel	27/06/07	9.276	9.3	5.4	7.5	4.3	0.55	17.449
Hain	Braine-le-Château	27/07/07	0.256	151.2	26.7	3.3	0.6	0.17	7.790
Herk	Wellen	06/07/07	0.450	18.4	2.5	0.7	0.1	0.13	1.666
Ieperlee	Zuidschotte	10/10/07	1.041	6.6	<1	0.6	–	–	1.393
Ijzer	Roesbrugge	10/10/07	13.203	<0.5	<1	–	–	–	–
Ijzer	Keiem	10/10/07	24.345	<0.5	<1	–	–	–	–
Kleine Nete	Grobbendonk	19/06/07	3.764	15.5	2.3	5.0	0.8	0.14	11.749
Leie	Deinze	02/08/07	2.068	5.1	<1	0.9	–	–	2.105
Leie	Menen	03/08/07	18.305	8.8	<1	14.0	–	–	32.603
Lesse	Gendron	11/10/07	5.610	<0.5	<1	–	–	–	–
Maas	Lanaken	12/10/07	60.000	18.9	2.0	5.1	0.5	0.10	227.884
Maas	Maaseik	11/10/07	60.000	12.2	1.1	63.3	5.7	0.07	147.516
Ourthe	Tabreux	12/10/07	7.668	<0.5	<1	–	–	–	–
Petite Gette	Opheylissem	17/07/07	0.535	22.4	4.0	1.0	0.2	0.17	2.410
Rivierbeek	Oostkamp	02/08/07	0.316	5.0	<1	0.1	–	–	0.316
Sambre	Solre	12/10/07	5.330	3.5	<1	1.6	–	–	3.799
Semois	Membre	18/10/07	4.946	<0.5	<1	–	–	–	–
Semois	Chiny	18/10/07	2.809	<0.5	<1	–	–	–	–
Senette	Ronquières	27/07/07	0.135	53.0	7.4	0.6	0.1	0.13	1.440
Viroin	Treignes	12/10/07	1.876	<0.5	<1	–	–	–	–
Zeeschelde	Melle	02/08/07	41.500	10.8	<1	38.6	–	–	89.974
Zenne	Lot	09/07/07	3.652	69.0	13.0	23.3	3.7	0.18	50.601
Zenne	Vilvoorde	09/07/07	5.555	520.2	114.9	249.7	55.2	0.21	581.665
Zuunbeek	Sint-Pietersleeuw	09/07/07	0.050	75.1	14.4	0.3	0.1	0.18	0.756

BE = benzoylcegonine, COC = cocaine, COC eq = cocaine equivalents, nm = not measured. The COC eq was calculated based on the following formula:  $\text{COC eq} = 2.33 \times \text{loads BE}$  as proposed by Zuccato et al. (2005).

Every WWTP was sampled twice, on Wednesday and on Sunday, with the purpose to investigate differences in the abuse of cocaine throughout the week. Cocaine abuse was significantly higher ( $p = 0.018$ , paired samples  $t$ -test) during the weekend compared with the week (the average difference was 0.08 g/day per 1000 inhabitants). For some WWTPs, there were no clear differences between the COC equivalents calculated between the two days and this could be explained by a wide variety of factors. For instance, for Mouscron WWTP we received a 99-h composite sample, which means that waste water from four consecutive days was collected, compared to the 24-h samples collected for the other WWTPs. Due to expected tourist activities at the North Sea, the concentrations of BE and COC in WWTP samples from Oostende and Heist are susceptible to large fluctuations which strongly depend on holiday periods. For the other WWTP samples, we could not find a proper explanation, but following factors could play a role: (i) local parties or small festivals; (ii) size and location of the WWTP; (iii) more regular cocaine consumers than recreational users.

The highest COC equivalents can be found in Antwerpen-Zuid, Deurne and Brussel-Noord WWTPs, with weekend COC equivalents of 1.8, 1.2, and 0.9 g/1000 inhabitants, respectively.

For the city of Antwerp, we have sampled three WWTPs (Fig. SI-5). The Antwerpen-Zuid and Deurne WWTPs receive waste water

from the centre of Antwerp and from surrounding suburbs, while Antwerpen-Noord WWTP receives only waste water from the northern suburbs (Fig. SI-5). As expected, higher COC equivalents were calculated in the waste water samples from Antwerpen-Zuid and Deurne WWTPs (Table 1). This is most probably due to two reasons: (i) these WWTPs receive waste water from the centre of Antwerp and (ii) a different social standard, more reported criminality and drug traffic in the suburbs whose waste water ends up in Antwerpen-Zuid and Deurne WWTPs compared to Antwerpen-Noord WWTP.

For Brussel-Noord WWTP, four 24-h composite samples were collected within the same week, on Friday, Saturday, Sunday and the following Wednesday, to evaluate possible daily variations in the cocaine equivalents (Table 4). As expected, we observed a clear peak in the COC equivalents during the weekend. Since it takes approximately 24 h for water to run from the domestic site to a WWTP (personal communication, Alain Vandelanoot, Aquafin), it means that the cocaine abuse on Friday is detected in the samples collected on Saturday. After intake, COC can be measured in urine up to 6 h, while BE is excreted over a larger period (up to 96 h) (Hamilton et al., 1977; Jufer et al., 2000). Therefore, samples from Sunday would still contain traces of BE originating from cocaine abuse on Friday. This explains the increasing BE loads

**Table 3**  
Concentrations of BE and COC of other studies

Place	Type	Conc. BE (ng/L)		Conc. COC (ng/L)		Reference
Olona, IT	Surface	183		44		Zuccato et al. (2008)
Lambro, IT	Surface	50		15		Zuccato et al. (2008)
Po, IT	Surface	4		1		Zuccato et al. (2008)
Arno, IT	Surface	22		2		Zuccato et al. (2008)
Thames, UK	Surface	13		4		Zuccato et al. (2008)
Po, IT	Surface	25		1		Zuccato et al. (2005)
Taff, UK	Surface	78		2		Kasprzyk-Hordern et al. (2008a)
Ely, UK	Surface	23		<0.3		Kasprzyk-Hordern et al. (2008b)
Broadmeadow, RI	Surface	nd		25		Bones et al. (2007)
Llobregat, ES	Surface	77		6		Huerta-Fontela et al. (2007)
BE	Surface	Min	1	Min	1	Present study
		Max	520	Max	115	
		Mean	37	Mean	7	
		Median	11	Median	2	
		SD	85	SD	18	
Ringsend, RI	Waste	290		489		Bones et al. (2007)
Spain, ES	Waste	810		79		Huerta-Fontela et al. (2007)
Nosedo, IT	Waste	1132		421		Castiglioni et al. (2006)
Lugano, CH	Waste	547		218		Castiglioni et al. (2006)
Cagliari, IT	Waste	640		83		Zuccato et al. (2005)
Cuneo, IT	Waste	420		76		Zuccato et al. (2005)
Latina, IT	Waste	750		120		Zuccato et al. (2005)
Cilfynydd, UK	Waste	1229		526		Kasprzyk-Hordern et al. (2008a)
Varese, IT	Waste	390		42		Zuccato et al. (2005)
BE	Waste	Min	33	Min	10	Present study
		Max	2258	Max	753	
		Mean	497	Mean	146	
		Median	351	Median	110	
		SD	440	SD	141	

IT: Italy, UK: United Kingdom, RI: Republic of Ireland, ES: Spain, BE: Belgium, CH: Switzerland.

throughout the weekend and the peak in COC loads observed on Saturday.

The concentrations and ratios of COC and BE observed in the present study are comparable to the results from Italian (Castiglioni et al., 2006; Zuccato et al., 2005, 2008), Spanish (Huerta-Fontela et al., 2007), and UK WWTPs (Kasprzyk-Hordern et al., 2008a,b; Zuccato et al., 2005) (Table 3). As hypothesized from the urinary

excretion pattern, higher BE concentrations than COC concentrations were found in waste water. However, Bones et al. (2007) measured higher levels of COC than BE in Irish waste water, which conflicts with the normal expected pattern resulting from the metabolism of cocaine.

The mean  $\pm$  SD ratio COC/BE in waste water samples was  $0.28 \pm 0.07$  and no outliers were observed. This ratio is comparable with the mean value (0.23) resulting from averaging the percentages of urinary excretion of BE and COC (Ambre et al., 1984, 1988; Cone et al., 1998). The calculated RSD (26%) is a consequence of the broad ranges of urinary excretion of COC and BE reported in the literature and of the differences in the stability of COC and BE in aquatic systems (Gheorghe et al., 2008). Yet, the RSD for waste water is lower than for surface water, indicating that for surface water, more factors, such as weather conditions, time of residence and turbidity, are responsible for the observed variations. The ratio COC/BE in waste water was higher than in surface water. The longer presence of COC and BE in surface water and the instability of COC in water are

**Table 4**  
Results for WWTP Brussel-Noord

Day	Conc. BE (ng/L)	Conc. COC (ng/L)	Loads BE (g/day)	Loads COC (g/day)	COC eq (g/day)	COC eq/1000 inh. (g/day)
Fri 14/12/2007	856	221	232.3	59.9	601.3	0.707
Sat 15/12/2007	1157	362	292.7	91.5	773.5	0.910
Sun 16/12/2007	1291	348	327.0	88.0	849.9	0.999
Wed 19/12/2007	909	274	222.7	66.8	585.6	0.689

BE = benzoylecgonine, COC = cocaine, COC eq = cocaine equivalents, inh = inhabitants.

possibly an explanation for this observed difference. Such difference between the COC/BE ratios in waste and surface water samples agrees with the difference observed by Zuccato et al. (2005).

#### 4. Conclusions

The present study offers for the first time a deeper insight in the daily/weekly variation of COC and its major metabolite, BE, in a large number of waste and surface water samples collected throughout whole Belgium. The detection of COC and BE in all waste water samples indicates a widespread abuse and emphasizes that such monitoring is important to get a better view on the growing issue of cocaine. The present approach of estimating cocaine use could be executed on a regular basis for short term (monthly) to long term (yearly) monitoring and gives an immediate and clear picture of the geographical variation in cocaine abuse. This information will ultimately serve to setup prevention campaigns in the regions with high cocaine abuse, while the regular analysis of waste water can quickly evaluate the effect of these prevention campaigns. Future assessments of cocaine abuse through analysis of waste water should include more accurate mathematical models which need to include various factors such as urinary excretion profiles, stability of COC and metabolites in water and adsorption on solid particles.

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#### Appendix. Supplementary data

Results of waste water samples taken on Wednesdays are visualised in Fig. SI-1. Sampling locations and results of surface water are presented in Fig. SI-2. Details and results of the method validation and the interlaboratory test are described in Tables SI-1 and SI-2, respectively. Quality control charts for COC and BE are given in Figs. SI-3 and SI-4, respectively. Fig. SI-5 gives a geographical situation of the three sample WWTPs from Antwerp. Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envpol.2008.07.020.

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